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SYMPOSIUM

ALERGIA E INMUNIDAD

ALLERGY AND IMMUNITY

ALLERGIE ET IMMUNITE

ALLERGIE UND IMMUNITAT

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INTRODUCTION:

IMMEDIATE AND DELAYED ALLERGY. DIFFERENCES BETWEEN BOTH

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Hypersensitivity reactions which take place in tissues have been classified into immediate and delayed according to the time required for the reaction to occur after the injection of antigen. Immediate hypersensitivity can be further subdivided into anaphylactic reactions and Arthus phenomena. These three types of immune reactions which can be elicited in sensitized animals by the same antigen have fundamentally different mechanisms involving different antibody types and cellular components. Contrasting with our relative ignorance of the precise mechanism of delayed hypersensitivity reactions, the immunological and pathogenic mecanisms of immediate hypersensitivity reactions are now clearly understood. Our knowledge of these reactions has been advanced by the studies of their passive transfer to normal recipients with known amounts of well characterized antibody, by methods initially introduced by KABAT [1].

I. *Anaphylactic Reactions.*

Local anaphylactic reactions which have been well studied in man [2] and many experimental animals, guinea pigs [3], dogs [4], rats [5], mice [6], [7], occur within minutes of the injection of antigen in sensitized animal. They are characterized by a sudden increase in vascular permeability and by the exudation of serum proteins into the tissues, brought about by the liberation of vasoactive amines such as histamine or 5-hydroxytriptamine released from mast cells by the reaction of antigen with tissue bound antibodies. It has been known for a long time that in man a special antibody type, called reagin, was responsible for these reactions [2], while human γ_2 antibodies with the same immunological specificity acted as blocking antibodies by competing for antigen. Human reagin was found to be thermolabile, to migrate as a slow β globulin [8], to sediment with a velocity somewhat greater than 7S [9] and to remain bound for weeks to sites of injection in the skin. Evidence has been also recently presented suggesting that human reagin is a β_{2A} glo-

bulin [10], [11]. In the past few years, new antibody types which mediate anaphylactic reactions in experimental animals in a manner analogous to human reagin in man, have been isolated and characterized. Guinea pigs [12], [13] and mice [7] each produce an antibody type migrating as a fast gamma globulin, which has been named γ_1, and which transfers anaphylactic reactions in its respective species. Unlike human reagins, γ_1 anaphylactic antibodies are produced by guinea pigs and mice in sufficient amounts to allow isolation and characterization. These γ_1 anaphylactic antibodies have 7S sedimentation constants, are heat stable, and can be distinguished from β_{2A} globulins. They do not fix hemolytic complement in the presence of antigen [14],[7]. Guinea pig and mice anaphylactic antibodies do not bind to the tissues of their respective species for as long a time as human reagins. Dogs [4] and rats [5] produce also a special antibody type which transfers anaphylactic reactions in its respective species. Both migrate as slow β globulins. The rat anaphylactic antibody has properties very similar to those of human reagin. It is present in very low concentration in the serum, is heat labile, has a sedimentation constant between 7S and 19S and sensitized rat tissue for extended periods of time [15]. It is now well established that each species possesses a distinct antibody type which is responsible for anaphylactic reactions within the species.

There is considerable evidence that anaphylactic antibodies must be bound to certain specific tissue receptors in order to sensitize for anaphylaxis. Most of the experiments have been made with passive cutaneous anaphylaxis (PCA) in the guinea pig, but the results obtained have been verified also with the anaphylactic antibodies of other species. Passive anaphylaxis requires a latent period of sensitization which is inversely proportional to the dose of antibody injected [16], which is believed to be the time required for the antibody to become bound to tissue receptors. The latent period for PCA varies with different animals 2 hours in the mouse. The extent of the latent period appears also to be related to the length of time which the skin remains sensitized. Other evidence for tissue fixation of anaphylactic antibodies is provided by experiments on competition of passive sensitization. Sensitization of guinea pigs for PCA by a specific antibody can be inhibited by an excess of γ_1 globulins not specific for the antigen used, which is believed to compete with the specific antibody for the available tissue binding sites [13]. The tissue sites where anaphylactic antibodies are bound have not been identified, probably because of the very small amounts of antibodies required for anaphylactic sensitization.

If attempts are made to sensitize a given animal species with the anaphylactic antibodies of another species, such as human reagins in guinea pigs or in rats [17], [5], or mouse γ_1 antibodies in guinea pigs [7], no sensitization is observed. Therefore, the receptors for binding to tissues differ in anaphylactic antibodies of different animal species. But, paradoxically, certain animal species can be sensitized for anaphylaxis with antibodies of foreign species. Taking the guinea pig as an example,

passive anaphylactic sensitivity can be transferred to guinea pigs with human, rabbit, monkey, dog [18], or mouse antibodies [7]. However, in every case the foreign antibody which sensitizes guinea pigs for anaphylaxis is not the anaphylactic antibody of the donor animal species, but rather the γ_2 complement binding antibody type which appears to possess by chance on its H chain the structure for fixation to guinea pig tissues which is characteristic of guinea pig γ_1 anaphylactic antibodies.

The conclusion can be drawn from this discussion that vasoactive amines are released from mast cells as a consequence of the combination of antigen with certain antibody types, not capable of fixing hemolytic complement, but bound to certain specific tissue receptors. The question remains to be answered: Is the formation of large immune aggregates required or is the simple bridging of two adjacent cell bound antibodies by one molecule of antigen sufficient to trigger the mechanism which causes the release of vasoactive amines from mast cells? This last hypothesis is consistent with the early experiments of KABAT and BENACERRAF [19], who showed that anaphylactic sensitization of guinea pigs could be obtained just as efficiently with "univalent" nonprecipitating as with precipitating rabbit antibody, provided antigen was in excess. It was proved beyond any doubt by the recent independent experiments of Eisen, Levine and Ovary, and their collaborators, who showed 1) that PCA reactions in guinea pigs sensitized with antibodies against the 2,4-dinitrophenyl hapten (DUP) could be provoked by a bivalent small molecular weight hapten, Bis-DNP Lysine [20], 2) that PCA reactions of equivalent intensity were elicited in sensitized guinea pigs by equimolar concentrations of antigen bearing 2 or more haptenic groups [21], 3) that antiovalbumin rabbit γ_2 antibody molecules made univalent *in vitro* by splitting the H chain's common SS bonds, and hybridized with normal γ-globulin by NISONOFF [22], were able to sensitize guinea pigs for PCA just as efficiently as intact bivalent antiovalbumin molecules [23].

In summary, one can conclude that the immunological events of the anaphylactic reaction are now well understood. It is a reaction mediated in each mammalian species by a special antibody type which is capable of binding to certain specific tissue receptors because of structural characteristics of its H chain. The release of vasoactive amines from mast cells though complex enzymatic steps, is triggered by the simple bridging of two antibody molecules by a single antigen molecule. An anaphylactic reaction lasts a short period of time and the tissue returns to normal without permanent damage; it can be considered to be a physiological process to trigger the vascular component of inflammation in response to invasion of the tissue by foreign organisms bearing antigens against which anaphylactic antibodies have been produced.

II. *The Arthus Reaction.*

The Arthus reaction is a completely different phenomenon from anaphylaxis in every respect. It can be best studied in an isolated form by passive sensitization since an actively sensitized animal may react to

the intradermal injection of an antigen with a complex reaction comprising anaphylactic, Arthus and delayed components. The passive Arthus reaction has identical features in rabbits [24], guinea pigs [25], and rats. It is characterized by a hemorrhagic necrosis of the skin which occurs 1 to 4 hours after the injection of antigen in an animal previously sensitized by a suitable amount of precipitating complement-fixing γ_2 antibody. A reverse passive Arthus reaction can be elicited if the antibody is injected intradermally and the antigen intravenously. The pathogenic mechanism of the reaction has been thoroughly investigated. Arthus reactions can only be obtained with precipitating antibody [26]. Non-precipitating rabbit antibody capable of transferring passive anaphylactic sensitivity to guinea pigs cannot provoke Arthus reactions [25]. Hemorrhagic Arthus reactions cannot be produced with guinea pig γ_1 antibodies which do not bind complement, in spite of the fact that this is the only antibody type which mediates anaphylactic reactions in this species [14]. Passive Arthus reactivity can only be transferred with γ_2 complement fixing antibodies. Hence, precipitating antibody and hemolytic complement are essential immunological components of the Arthus reaction. It is clear that in the guinea pig different antibody types are responsible for anaphylaxis (γ_1) and for Arthus reactions (γ_2). Furthermore, the amounts of antibody required to transfer these reactions passively in guinea pigs is very different, 0, 01 μg AbN for PCA and 10 μg AbN for reverse passive Arthus.

The initial pathogenic event in the Arthus reaction is the formation of an immune precipitate of antigen, antibody, and complement within the wall of the small blood vessels by circulating antibody and tissue antigen diffusing towards each other. This precipitate can be identified by immunofluorescence under the endothelial cells next to the basement membrane [27]. The immune precipitate by itself does not cause tissue damage, but it causes the accumulation of polymorphonuclear leucocytes and platelets [28]. The leucocytes phagocytize the complexes and release their proteolytic lysosomal enzymes. Platelets also accumulate and a thrombus is formed. The vessel wall is destroyed and hemorrhagic necrosis of the tissue follows. Unlike anaphylactic reactions, the Arthus reaction cannot be considered to be a physiological mechanism, but rather a form of immunologically induced tissue damage requiring repair for healing. In spite of the striking differences in the mechanisms of Arthus and anaphylactic reactions which have been summarized in Table I, they possess certain similarities which differentiate them both from delayed hypersensitivity reactions. They can both be transferred passively by serum antibodies; they occur from within a few minutes to a few hours after the injection of antigen; they have the same type of immunological specificity which differs strikingly from that of delayed hypersensitivity reactions.

When using hapten immunological systems such as conjugates of the 2,4-dinitrophenyl group with bovine gamma globulin (DNP-BGG), guinea pig albumin (DNP-GpA) or ovalbumin (DNP-Ova), anaphylactic and Arthus reactions have been shown to require only the immunological

specificity of the hapten and the first amino acid (lysine) to which it is attached [30], [30]. Thus reactions of equivalent intensity are elicited by DNP-BGG, DNP-GpA, or DNP-Ova bearing comparable numbers of haptenic groups, in animals sensitized with DNP-BGG, showing the lack of carrier protein specificity of the reactions of immediate hypersensitivity in contrast with the marked carrier protein specificity requirements of delayed hypersensitivity reactions. As a rule immediate hipersensitivity reactions parallel closely the immunological specificities of *in vitro* antigen-antibody reactions which in the case of hapten protein system, show also a lack of carrier protein specificity.

III. *Delayed Hypersensitivity.*

Delayed hypersensitivity reactions are immunologically specific, inflammatory reactions which take 12 or more hours to reach their maximal intensity. These reactions were thought at first to be characteristic of the proteins of the tubercle bacillus and of other infectious agents, but it was shown that delayed reactions could be obtained with all protein antigens, in species capable of exhibiting this type of reactivity such as man, guinea pigs, rabbits and rats, especially if the immunizing antigens were mixed with mycobacteria [31]. The histological features of delayed reactions are vasodilatation of small vessels and massive perivascular infiltration with mononuclear cells which have the morphological appearance of large lymphocytes or small monocytes [32]. There is no hemorrhage or severe edema as in the Arthus reaction. Delayed hypersensitivity reactions can be elicited in sensitized guinea pigs with the same antigens which can also provoke anaphylactic or Arthus reactions. The most thorough studies have been made with hapten protein conjugates such as picryl guinea pig albumin. Using suitable immunizing doses of such antigens in complete Freund's adjuvant, delayed hypersensitivity reactions can be observed as early as 5 to 9 days after immunization, when serum antibodies, γ_1 or γ_2, which transfer immediate hypersensitivity reactions, are not present in the circulation in appreciable amounts [33], [34]. Moreover, delayed hypersensitivity in the absence of circulating antibody can be observed for weeks when guinea pigs are immunized with antigen antibody complexes in antibody excess in complete Freund's adjuvants [35]. This technique diminishes the intensity of the antigenic stimulation. Similarly delayed hypersensitivity to the carrier protein in the presence of a high concentration of antihapten antibodies is observed when guinea pigs are immunized with heavily conjugated proteins such as picryl BGG [33].

Contrasting with the transfer of immediate hypersensitivity reactions with serum antibodies, delayed hypersensitivity reactions cannot be transferred with antiserum from sensitized animals, regardless of its antibody content [31].

The experiments of CHASE and LANDSTEINER have demonstrated that these reactions can be easily transferred with peritoneal exudate cells,

lymph node or spleen cells of sensitized animals [36, 37]. Another distinctive feature of delayed hypersensitivity reactions which distinguish them from serum antibody mediated hypersensitivity reactions is their remarkable immunological specificity. Comparative studies of the immunological specificities of delayed and immediate reactions have been made in guinea pigs with hapten conjugates of a nonantigenic homologous protein such as picryl guinea pig albumin. Delayed hypersensitivity reactions required in all cases the immunological specificity of the carrier protein as well as that of the hapten [29, 38]. Thus guinea pigs sensitized picryl Gp Alb demonstrated delayed reactions only to the immunizing picryl conjugate but showed immediate hypersensitivity reactions, anaphylaxis and Arthus to picryl conjugates of other immunologically unrelated proteins. A similar degree of immunological specificity is observed in contact reactions which have been shown to be delayed reactions with the immunological specificity of the chemical reactant and of a variety of host proteins with which it combines *in vivo* at the time of immunization and testing [30, 39].

The reason for the high degree of immunological specificity of delayed hypersensitivity reactions has not been precisely understood. The possibility has been considered that stronger binding forces are required to elicit such phenomena which depends upon the reaction of antigen sensitized cells with antigen than are required for the reaction of antigen with free or cell bound antibodies. Such stronger binding forces could be contributed by the carrier protein to the specificity of a hypothetical cellular antibody. Another hypothesis would postulate the existence of another mediator other than γ-globulin for delayed hipersensitivity reactions.

It is of interest to note that a similar high degree of immunological specificity has been observed in other reactions of sensitized cells with antigen in the case of anamnestic or secondary response. Rabbits sensitized with hapten protein conjugates show a secondary response only when injected with the same hapten on the identical carrier protein [40]. The high degree of immunological specificity observed in delayed reactions appears therefore to be characteristic of the reaction of all actively sensitized cells with antigen.

The marked differences between immediate and delayed hypersensitivity reactions have been listed in Table II.

The fact that delayed reactions can only be transferred by sensitized cells and that the reactions are characterized by cellular infiltrates of mononuclear cells suggested that the transferred cells are reacting at the site of injection of the antigen. Experiments were conducted in various laboratories to explore this possibility using cells lebelled with H^3-thymidine to transfer delayed hypersensitivity.

The results from 3 laboratories agreed that no specific arrival of sensitized cells could be demonstrated at the site of the reactions [41, 42, 43]. However, NAJARIAN and FELDMAN reported that they observed a small but significant number of labelled cells in specific delayed reactions [44, 45].

TABLE I

DIFFERENCES BETWEEN PCA AND REVERSE ARTHUS REACTIONS IN GUINEA PIGS

	Time of Reaction	Latent Period	Antibody Type & Amount Needed	Immune Aggregation	Tissue Bound Antibody	Hemolytic Complement
PCA	10-15 min	3-5 hrs	γ 1 0.01 AbN	Not Required	Essential	Not Required
Arthus	1-2 hrs	None	γ 2 10.0 AbN	Essential	None	Essential

	PMN Leucocytes & Platelets	Vasoactive Amines	Antihistamine	Nitrogen Mustard	Heparin
PCA	Not Required	Essential	Inhibited	Not Affected	Not Affected
Arthus	Essential	Not Required	Not Affected	Inhibited	Partially Inhibited

TABLE II

DIFFERENCES BETWEEN IMMEDIATE AND DELAYED HYPERSENSITIVITY REACTIONS

	Time of Reaction	Transfer by Antiserum	Transfer by Immune Cells	Immunological Specificity	Carrier Protein Specificity	Response of Agammaglobulinemic Patient
Immediate	15 min-2 hrs	Positive	Positive	Smaller	Absent	No Response
Delayed	12-48 hrs	Negative	Positive	Greater	Essential	Weakened but Present

The experiments of McCLUSKEY and BENACERRAF, who labelled the recipient rather than the donor guinea pigs with H^3-thymidine, revealed that about 80 percent of the infiltrating cells in the lesions were then labelled, demonstrating that the great majority of the cells in transferred delayed hypersensitivity reactions were contributed by the host and had been recently synthesizing DNA [41]. It would appear therefore that if the donor animal did contribute actively sensitized cells to trigger the reaction they were a relatively small number.

The identity of the cells which constitute the infiltrate in delayed reactions has been studied recently with the electron microscope. They were shown to consist in their majority of young macrophages or monocytes which originate from the circulation [46]. These observations suggest that macrophages may be the effector cells in delayed hypersensitivity reactions [47] and that the reaction is elicited by the action of very few actively sensitized cells or by the transfer from actively sensitized cells of some factors to the other infiltrating cells.

These conclusions are consistent with the *in vitro* observations made with cells from sensitized guinea pigs. These cells obtained from peritoneal exudates, or from spleen or lung, fail to migrate *in vitro* in the presence of specific antigen [48]. The immunological specificity of this *in vitro* reaction, studied with hapten-protein conjugates, show marked carrier protein specificity as in the case of delayed skin reactions. The cells that fail to migrate have the morphology of macrophages [48, 49]. Furthermore, if 5 percent of the cells from sensitized animals are mixed with 95 percent of nonsensitized cells, all the cells fail to migrate in the presence of antigen, showing that under these conditions a few sensitized cells can affect the behavior of all the cells in the system [48].

Another important feature of delayed hypersensitivity reactions which deserves to be discussed is peculiar to these reactions in man, where delayed hypersensitivity has been transferred by LAWRENCE with cell-free extracts of circulating leucocytes from sensitized donors [50]. The "transfer factor" active in man, whose chemical nature has not been identified as yet, has been found to be able to diffuse through a dialysis membrane. Transfer with cell-free extract of sensitized cells has not been successful in experimental animals.

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ANTIBODIES IN ALLERGY

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INTRODUCTION.

A consideration of the rôle played by antibodies in the development of allergic reactions requires a previous assesment of the concept of allergy. Although it would be highly desirable that the word *allergy* would recover the original sense given to it by von Pirquet to describe the biological state following contact with an antigen, and to use the words *hypersensitivity* and *immunity* to refer to the two orders of clinical manifestations which can derive from it, on the present occasion, and for the sake of convenience, we shall use allergy as synonimous to hypersensitivity, as it is the most frequent usage, thus considering as allergic those reactions immunologic in nature which can produce functional or structural changes deleterious to the subject on which they develop.

Allergic reactions obey to various basic mechanisms, according to which they have been grouped by COOMBS and GELL [1] in four types which are listed, with their more prominent features, in Table I.

TABLE I.—CLASSIFICATION OF ALLERGIC REACTIONS

According to Coombs and Gell (1)

Type I. (ANAPHYLAXIS).

Tissue cells passively sensitized by antibody produced elsewhere.
Release of pharmacologically active substances.

Type II (CYTOLYTIC OR CYTOTOXIC REACTIONS).

Antigen is a component of tissue cells.
Antigen or hapten has become intimately associated with tissue cells.

Type III (ARTHUS REACTION, SERUM SICKNESS, ANTIGEN-ANTIBODY COMPLEX SYNDROME).

Potentially precipitating antibody (not fixed to tissue cells).
Reaction takes place in tissue spaces or in the blood stream.

Type IV (DELAYED, TUBERCULIN-TYPE REACTION).

Specifically modified mononuclear cells.
(Humoral antibody not involved.)

ANAPHYLAXIS.

All the forms of anaphylaxis known, systemic anaphylactic shock as well as local reactions either *in situ* or in isolated organs, both active and passive, are exactly comparable to each other from the point of view of the antibodies involved, and for that reason a further division of the different types of anaphylaxis will not be attempted here. Much of what we know about the antibodies which cause anaphylaxis has been learnt from experiments on passive anaphylaxis in isolated organs or at restricted areas of the skin of experimental animals. It should be remembered, as COOMBS and GELL [1] point out, that even in the actively sensitized animal or person its cells are passively sensitized by way of the circulation and tissue fluids.

Contrary to the Arthus reaction, it is not necessary for the development of anaphylaxis that the antibody be of the precipitating type. KABAT and HENAGERRAF [2] have shown that co-precipitating antibody is as effective as precipitating antibody in producing anaphylaxis. OSLER [3], on the contrary, has obtained data suggesting that non-precipitating antibody, although able to produce anaphylaxis, is less effective than precipitating antibody in this respect. Atopy, a special form of anaphylaxis, is due to a type of non-precipitating antibody, the so-called reagin, which will be discussed later on.

For an antibody to cause anaphylaxis it is a necessary requisite that it can fix to the cells of the tissue to be sensitized. This is true in man and in all the animal species studied, except in the rabbit, in which fixation apparently takes place only to the formed elements of the blood [4]. The need for fixation manifests itself in the occurrence of a latent period in passively sensitized animals between injection of the immune serum and sensitization of the tissues. This interval varies inversely with the concentration of antibody; it becomes practically nil when the antibody is given in very great amounts. In the rabbit no latent period is needed for passive sensitization to systemic anaphylactic shock, which is the only form of anaphylaxis which can be passively produced in this animal.

The ability to fix to tissue cells is a property of a part of the gamma globulin of some, not all, animal species. Thus, passive anaphylactic sensitization can be produced in the guinea pig using guinea pig, rabbit, dog, monkey and many human antibodies, but not using horse, chicken or ruminant antibody. Fixation does not depend on the antibody specificity of the gamma globulin used; even "normal" gamma globulin fixes to the tissues, in such a way that antibody and normal gamma globulin present in the same serum compete with each other for fixation to the tissues. Passive sensitization of a site of guinea pig skin can be prevented by the previous injection at the same site of normal gamma globulin of the same or different animal species as the antibody to be injected later, provided it comes from one of those species which have been said to be able passively to sensitize guinea pig tissues.

Not all the immunoglobulins of the species whose sera can be used for passive sensitization are able to sensitize. 19S antibodies (corresponding in man to beta-2M globulins) fail to fix to guinea pig tissues and therefore do not cause anaphylaxis in this species. The same happens with human beta-2A globulin [5], as it will be seen in more detail on discussing atopic reagins. But even in the case of the antibodies contained in gamma (gamma-2; 7S gamma) globulin, the sensitizing ability is not uniform [6]. BENACERRAF et al. [7] have extensively studied two kinds of 7S antibodies in the guinea pig, referred to as 7S-gamma-1 and 7S-gamma-2, according to their electrophoretic mobilities. While 7S- gamma-2 antibodies fix complement and are very active in the production of Arthus reactions, they do not sensitize guinea pig tissues; 7S-gamma-1 antibodies, on the other hand, have little activity as far as the production of Arthus reactions is concerned, and do not fix complement, but they are able passively to sensitize the skin and organs of the guinea pig. Similar observations have also been made by WHITE, JENKINS and WILKINSON [8]. These properties seem to give 7S-gamma-1 antibodies some similarities with human reagins, but, as opposite to the latter, guinea pig 7S-gamma-1 antibodies pass from mother to foetus [9]. The transfer of anaphylactic sensitivity from mother to foetus has also been studied in our laboratory [10]; it is another example of passive sensitization, taking place in animals born even 15 months after a single injection of antigen to the mother. The passage of antibody to the progeny depends on the structure of fragment III of its molecule.

The mechanism by which antibody fixes to cells is not well understood. The union is firm, but not irreversible, antibody being slowly eluted by other gamma globulins [11]. Fixation takes place through the fragment III obtained by digestion of the gamma globulin molecule following Porter's method, as shown by the fact that only using this fragment and not fragments I or II as antigens can reversed passive anaphylaxis be obtained in the skin [12].

Reversed passive anaphylaxis requires that the antigen, given before the antibody, can fix to the tissues. It can only be produced, therefore, when the antigen is a gamma globulin (or the fragment III of its molecule) which can cause direct anaphylactic reactions. The origin and kind of antibody is immaterial in this case, as its fixation to tissue cells is not necessary: 19S antibodies or antibodies [13] from "inadequate" species (horse, sheep, hen, etc., in the guinea pig) will "work" [14].

ATOPY.

Atopy, a special form of anaphylaxis, is characterized by the presence of a peculiar type of antibody, called atopic reagin, skin sensitizing antibody or P. K. antibody (responsible for Prausnitz-Küstner passive transfer tests). Other antibodies (blocking, haemagglutinating, precipitating, etc.) present in atopic patients will not be considered here in detail, as they have not been proven resposible for any of the clinical

manifestations of atopy, even when they may be useful for the diagnosis or from the point of view of treatment. They will probably be dealt with more extensively tomorrow by Dr. Sherman or some of the participants in the afternoon panel meeting.

It is hard to doubt at present that reagin is the antibody responsible for atopic reactions. In addition to the now classic findings of RAMÍREZ (cited by VOORHORST [15]) and LOVELESS [16] on the occurrence of generalized passive sensitization by the blood of atopic patients, recent studies by CONNELL and SHERMAN [17], [18] show a narrow relationship between reagin titre and the frequency and severity of symptoms. Moreover, most symptom-free people having cutaneous reactivity sooner or later develop typical atopic symptoms [19]. In favour of this opinion is also the fact that the occurrence of constitutional reactions following hyposensitizing therapy is related to the initial titre of skin sensitizing antibody[20], [21], [22].

The chemical nature of reagins has been the subject of much study and controversy. Depending in part on the method used, they have been ascribed to any of the electrophoretic fractions of serum proteins [23], even to albumin [24]. In 1957 HUMPHREY and PORTER [25] pointed out that reagins are contained in a chromatographic fraction of gamma globulin also present in normal sera, having the electrophoretic mobility of gamma-1 globulin. AUGUSTIN and HAYWARD [26], [27] and STANWORTH [28] identified reagins as fast moving gamma globulins of normal molecular weight (not macroglobulins) and stressed their tendency to complex with other proteins, particularly alpha-2 macroglobulin. AUGUSTIN [29] could remove the reaginic activity from a serum by precipitation with an anti-gamma globulin serum which, nevertheless, had not been previously absorbed with other immunoglobulins. The molecular weight of reagin, as judged from its sedimentation constant in the ultracentrifuge, was the matter of much debate, mainly between CAMPBELL and SEHON and their colleagues [30], [31], [32]. They would be 7S globulins according to the former, 19S globulins according to the latter; they also thought of the possible presence of both kinds of antibodies in the same serum, or of an antibody with an intermediate molecular size. ROCKEY and KUNKEL [33] included reagins in a class of antibodies with an intermediate sedimentation constant: 9S-15S, and called attention, in agreement with other workers [33], to their relative susceptibility to sulfhidrilic reagents. In Rockey and Kunkel's paper the idea was put forward that reagins could be beta-2A globulins.

In 1962 HEREMANS and VAERMAN [34] pointed to beta-2A globulin as the possible carrier of reaginic activity, firmly based on the results obtained by serum fractionation and on a comparison of some of the known properties of reagins with those of beta-2A globulin. YAGI, MAIER, PRESSMAN, ARBESMAN and REISMAN [35], [36], using radioimmunoelectrophoresis, got results which they interpreted as indicating that the reagins were contained in the beta-2A globulin and perhaps also in the beta-2M globulin of the sera studied. The antibody found in the spontaneous hypersensitivity of the dog, in many respects similar to human reagin, also seems to be a beta-2A globulin [37].

Confirmation of the beta-2A globulin nature of reagins was achieved by FIREMAN, VANNIER and GOODMAN [38] who, in very well controlled experiments, succeeded to remove the reaginic activity from the sera of three ragweed-sensitive patients by specific precipitation of beta-2A globulin with an anti-beta-2A globulin serum. Exactly the same results have been obtained by me using the sera of five patients sensitive to grass pollens (three patients), molds and foods, in a series of experiments which had to be discontinued when a normal subject (the present author) on which P. K. tests were performed developed serum sickness by sensitization to the proteins (including gamma globulin) of the rabbit serum used for precipitation of beta-2A globulin.

The knowledge of the chemical nature of reagin allows for an explanation of some of the peculiarities of this antibody to be given. The molecular weight of beta-2A globulin is 160.000, and its sedimentation coefficient 7S, but they often appear to be higher, due to the tendency of this protein to aggregate with itself and with other proteins. The electrophoretic mobility is rather heterogeneous, showing a maximum in the beta-2 zone and extending predominantly towards the anode, to reach the alpha-2 zone. This two facts, electrophoretic heterogeneity and aggregation with other proteins, help to explain why reaginic activity has been found in various electrophoretic fractions.

Atopic reagins do not sensitize the skin or other organs of the experimental animals usually employed in the laboratory. In the last few years LAYTON and co-workers [39, 40, 41, 42, 43] have extensively studied the passive transfer of sensitivity by means of human serum to the skin and mucous membranes of *Macaca irus* and some other monkeys; the antibody responsible for this transfer is in all likelihood the same reagin which sensitizes human skin. The failure to passively sensitize other animal species, particularly the guinea pig, is not surprising since, as it has been said before, human beta-2A globulin does not fix to the tissues of this animal [5, 44].

There is no unanimous agreement concerning the cells to which reagins get fixed: endothelial cells, mesenchimal cells and the epithelial cells of the skin and mucous membranes have been incriminated [15]. According to fluorescent antibody studies carried out by RAPPAPORT [45, 46], reagins are present, in the atopic patient as well as in passively sensitized sites of normal skin, in all the eptihelial cells of the skin, in macrophages and in pericytes.

The mechanism by which the union of reagins to tissue cells takes place has also been a matter of controversy. AUGUSTIN [47] suggested that reagins might be bispecific antibodies, with a combining group specific for the causative allergen and another one, similar in all reagins, specific for some antigenic determinat in tissue cells. More recently, STANWORTH [48] thinks that the union of reagins to the tissues, although antigen-antibody in nature, is not due to reagins being bispecific antibodies, but rather to the existence of a cross-antigenicity between some substance in the tissues and the antigenic determinants of the allergen.

Probably, there is an inverse relationship between the degree of predisposition and the strength of the allergenic stimulation needed to elicit an atopic response, in such a way that under the more frequent circumstances only people with a marked predisposition are able to develop reagins in quantity enough to produce clinical disease, while with more potent stimuli even individuals with little or no family history can respond in the same fashion. In favour of this opinion are, amongst others, the following observations: in serum sickness, as we shall see later, an antibody in many respects similar to, if not identical with, atopic reagin often develops, even in subjects without any previous personal or family history of sensitivities of this type [23]. During *Ascaris* infestations and after the injection of extracts of this worm, most people develop an atopic-type sensitization, with the presence in their sera of skin sensitising antibodies. Exposition to castor bean or injection of the allergen extracted from it also causes sensitization accompanied by the presence of reagins, even in people without allergic antecedents [65], [66]. The injection of emulsified pollen extracts induces in some non-atopic persons immediate cutaneous reactivity, in some cases accompanied by the presence of a circulating antibody with the properties of reagin [67], [68]. Recent findings of FARR [69] and of SALVAGGIO, CAVANAUGH, LOWELL and LESKOWITZ [70] seem to point to an increased permeability of mucous membranes to inhaled or ingested antigens as the fundamental trouble in atopic subjects.

PATERSON, PAGE and GOOD [71] studied the occurrence of atopy in patients with agammaglobulinaemia. In one case they observed the disappearance of the symptoms of hypersensitivity with the development of the disease. In a group of patients with agammaglobulinaemia the susceptibility to sensitization by *Ascaris* antigens was diminished. These findings are not surprising, since the decrease of gamma globulin is often accompanied by an equally marked decrease of beta-2A globulin. In other cases, despite the presence of severe hypogammaglobulinaemia, there was a state of atopic hypersensitivity. Other workers [72], [73] have reported cases in which atopic sensitivity was present in the absence of detectable amounts of beta-2A globulin from the serum. This does not mean that in such cases skin sensitizing antibody is a globulin other than beta-2A, since undoubtedly very small amounts of this antibody are sufficient to cause hypersensitivity. I could not find significant differences, except on rare occasions, between atopic and normal people as far as the concentration of beta-2A globulin in their sera was concerned.

Many things remain to be learnt about reagins, on the basis of recent knowledge of their nature. Other antibody activities bound to beta-2A globulin have been still insufficiently explored [74]. We do not know the reason and the significance of the elevated concentration of beta-2A globulin, out of proportion with other proteins, found in some body fluids such as saliva [75] and tracheobronchial secretions [76]; it must be in some way related with the finding that the concentration of reagin in nasal polips is far higher than that found in the serum of the same patient [77].

the presence of either allergen or the human antibody in the coprecipitate directly, by agar precipitation between the redissolved coprecipitate and the corresponding antiserum, or indirectly by agglutination or precipitation inhibition tests using the corresponding homologous systems, or finally by testing the capacity of the washed coprecipitate to actively sensitize the guinea pig for anaphylaxis to the immune reagent sought. While I obtained positive results on some occasions, I never reached the conviction that they were due to reagins.

From the ensemble of all these negative results it seems more and more likely that the failure to detect reagins *in vitro* is due to the fact that these antibodies do not form a sufficiently stable union with the antigen in the absence of cells. *In vitro* reactions involving cells apparently offer more promising results, such as those obtained measuring the release of histamine from peripheral blood cells on addition of the antigen [60] or those of the questionable mast cell degranulation test.

Another property of reagins not shared by most other antibodies is heat lability: reagins lose their skin sensitizing activity when they are heated at 56° C for periods from thirty minutes to four hours. Although it seems most likely that heat inactivation of reagins is due to destruction of their skin combining capacity, there are those who think that it is rather due to the inability of heated reagin to combine with allergen [20]. With respect to the lability of reagins, it has been observed that alpha-2 macroglobulin, a protein for which reagins show a marked affinity [26], [27], seems to play a protective role on reagin, so that the latter inactivates on being separated from it during serum fractionation procedures [28], [61]. It is interesting to note that this protective factor is itself heat labile [62]. Studies on the effects of heat upon some properties of beta-2A globulin are being carried out in our laboratory.

The failure of reagins to pass the placental barrier was one of the points taken some time ago in favour of the idea that skin sensitizing antibody might be a macroglobulin. As a matter of fact, it is simply a consequence of the beta-2A globulin nature of reagins; this globulin does not pass the placenta in significant amounts and therefore is almost completely absent from the blood of the umbilical cord and of the newborn during the first few weeks of life [63], [64]. The reason for such a behaviour must lie in the special configuration of the fragment III (polypeptide chains H) of its molecule, since in this fragment resides the ability of another immunoglobulin, gamma globulin, to pass through the placenta. Alternatively, it could be due to its tendency to form aggregates with itself and other proteins, or else to its affinity for epithelial cells, which would cause it to remain attached to the epithelial layer of the placenta.

It is generally admitted that the heredity of a special constitution would be necessary for the development of atopy and therefore for the production of this particular type of antibody, the atopic reagin, which could not be artificially stimulated. It is certainly a frequent clinical observation a familial tendency to the suffering of atopic diseases, but such a hereditary predisposition needs not be taken in an absolute sense.

It is interesting to note that the most important antigens in cytotoxic reactions are those which are constituents of or adhere to the surface of the cell, as most antibodies are generally unable to enter intact cells [87].

Arthus reaction.

The third type of allergic reactions includes Arthus reaction and serum sickness, in addition to comparable reactions that can be produced by the injection of antigen-antibody complexes formed *in vitro*.

The antibody in Arthus reaction must be potentially precipitating and present in the circulation at a rather high concentration; the severity of the reaction, other circumstances being the same, is proportional to the serum titre of precipitating antibody. No fixation of antibody to tissue cells occurs, and therefore it can be passively produced, even in the rabbit, using antibody of any animal species, without the need for a latent period between passive sensitization and antigenic challenge. For the same reason a passive reversed Arthus reaction can be produced using any antigen which can be precipitated by its antibody, although it fails to fix to the cells of the tissue in which the reaction takes place.

The fact that only precipitating antibody can produce typical severe haemorragic Arthus reactions suggests that a true antigen-antibody precipitation in the tissue spaces is necessary for the reaction to develop. While according to some [11] it is not certain whether the formation of an immune precipitate is necessary or not, we must admit that it is not an easy task to recognize the formation of a precipitate within a tissue. The concept of precipitation corresponds to a physical change occurring in a liquid mass, and it is not easy to find its equivalence to the conditions of observation possible in the tissue spaces. Habesin and Hansfield [88], however, in an electron-microscopic study of the Arthus reaction using ferritin as the antigen, have reported the finding of images in all respects comparable to those given by antigen-antibody precipitates prepared *in vitro*. As for the place where precipitation occurs, while according to some it would initially be in the walls and around the small vessels [89], according to others it would take place in the lumen of venules, later to go out to tissue spaces [89]. Possibly, as Fernando and Movat [90] point out, the localization depends to a great extent on the nature of the antigen and its capacity to diffuse through the vessel walls. In Oort and van Rijssel's opinion [91] the first event is a reaction in antigen excess in the intercellular spaces; soluble complexes are formed which cause an increase in vascular permeability, as a consequence of which more antibody leaves the circulation and causes precipitation of the antigen contained in the above complexes.

Serum sickness.

The antibody responsible for both clinical and experimental serum sickness is the one first formed after antigenic stimulation. Like in

Four years ago, COOKE, MENZEL and MYERS [78] suggested that reagin should be vaguely described as a "factor", more similar to a coenzyme, rather than as an antibody. Today, we cannot longer have any doubt that it is a true antibody: firstly, reaginic activity resides in a serum fraction which had been beforehand considered, for strong reasons, as an immunoglobulin [79]; secondly, it appears or increases after the injection of the specific antigen, as it has been repeatedly shown in studies of the antibody response after hyposensitization therapy [80], [81], [82]; and lastly, it reacts specifically with the antigen (allergen) which induced its production and cross-reacts with antigens having a certain relation to it; this reaction, although not demonstrable *in vitro* in the absence of cells, shows some rather precise quantitative relations [83].

CYTOTOXIC REACTIONS.

The reactions which occur when antibody reacts with an antigen forming part of a cell or intimately adhered to it are, as a whole, less definite and less homogeneous than other types of allergic reactions. While there is a number of perfectly characterized lytic reactions, some phenomena included in this group may be considered as a reversed anaphylaxis (for instance, in part, the reaction produced by the injection of anti-Forssman serum into an animal whose tissues possess this antigen) and some others bear much resemblance to reactions of the third type (for example, Masugi's nephrotoxic nephritis).

Almost any sort of antibody can be responsible for a cytolytic or cytotoxic reaction. Since very often cyto- or organotoxic reactions are mediated by the serum complement complex, it cannot be doubted that in such cases a fundamental property of the antibodies at play must be the ability to fix and activate complement. Cells acted upon by specific antibody and complement undergo lysis or at least become more susceptible to the action of certain enzymes [84], at the same time as its ability to bind amino acids diminishes [85]. But also non-complement fixing antibodies, even incomplete antibodies that cannot produce any of the classic *in vitro* effects may cause severe lesions and tissue damage, because agglutination or changes in the cell membrane brought about by certain antibodies are sufficient to cause the cell to be more readily attacked or eliminated.

In one of the clearest and most important examples of cytotoxic reaction, destruction of incompatible red cells *in vivo*, MOLLISON [86] has recently reviewed the various ways in which such destruction can take place, according to the *in vitro* properties of the antibody involved. Thus, *in vitro* haemolytic (complement fixing) antibodies cause intravascular haemolysis, while under the action of non-complement fixing (either agglutinating or incomplete) antibodies destruction takes place extravascularly, mainly in the liver or in the spleen, according to the potency of the antibody and its concentration.

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Arthus reaction, it is a complete, potentially precipitating antibody that can also be shown by haemagglutination and complement fixation tests and by passive cutaneous anaphylaxis in the guinea pig [92]. Antibody in serum sickness does not get fixed to the tissues, but reacts in the circulation with the antigen still present there. Antigen-antibody complexes in moderate antigen excess are thus formed, their presence in the circulation being detected at the time of the development of the typical symptoms of the disease. The severity of the symptoms keeps no relation to the amount of circulating antibody, but rather to that of complexes, although the parallelism between them is neither absolute nor constant [93]. It seems out of doubt that no true precipitation takes place in serum sickness; in fact, insoluble antigen-antibody complexes at equivalence do not cause serum sickness. A deposition of the complexes occurs in the vessel walls and in several organs, not due to specific immunologic appetence for these localizations, but to physical phenomena [94, 95, 96].

In addition to the former, a skin sensitizing antibody may also appear in human serum sickness, similar in many respects to atopic reagin, possibly with some differences [23, 92, 97].The skin sensitizing antibody of serum sickness has not been so extensively studied as atopic reagin, and therefore minor differences between them cannot be accorded too much value. Skin sensitizing antibody cannot be shown in every case, but may appear even in patients without previous history of allergy [98]. It does not follow the same course as precipitating antibody, but rather tends to appear a little earlier and last shorter than the latter. It makes its appearance at the time of the development of the main symptoms [98]. This reaginic antibody is likely to be responsible for some of the manifestations of serum sickness in man, especially the urticarial rashes, oedema and asthmatic attacks which may occur in the disease, while kidney lesions, arthralgia, lymphadenopathy and non urticarial exanthemata are caused by soluble antigen-antibody complexes, like in serum sickness artificially produced in experimental animals [98, 99].

ANTIGEN-ANTIBODY COMPLEXES.

The pathogenic role played by antigen-antibody complexes in hypersensitivity reactions cannot be doubted upon, since the biological activity of antigen-antibody complexes formed *in vitro* has become known to us (reviewed by WEIGLE [100] and by ISHIZAKA [101]). Thus, lesions comparable to those of either passive local Arthus reactions or serum sickness have been reproduced by the injection of such complexes in the skin of the rabbit or intravenously in the same animal, respectively. The antibody in such instances may come from the same or different animal species as the one used for the test, although heterologous antibody is less effective than homologous one [102]; it is not neccessary that the antibody can fix to the tissues of the host. *In vitro* preformed complexes are, in equivalent amounts, less active than complexes formed

in vivo (in serum sickness), probably because of their different distribution in the body or of the different quality of the antibody involved [103].

But apart from these reactions which mimic Arthus reaction and serum sickness, the biologic activity of antigen-antibody complexes formed *in vitro* can reproduce the other types of allergic responses which have been considered before. Thus, lytic effects have been observed on red cells, leucocytes and platelets, apparently depending on the union of the complex (through its antigen or its antibody moiety) to the surface of these elements; such effects are comparable to the cytotoxic reaction. The ISHIZAKAS, SUGAHARA and CAMPBELL [101] have extensively studied anaphylactic-type reactions produced by antigen-antibody complexes formed *in vitro*. These reactions can reproduce both systemic anaphylactic shock and local anaphylaxis on *in vitro* isolated organs or on the skin of susceptible animals. Reactions of this type are characterised by the release of pharmacologically active substances responsible for the symptoms, and by the need for fixation of the antibody (occasionally the antigen) molecule to tissue cells, because of which only antibodies of some animal species may cause the reactions in a given animal, in the same way as in anaphylaxis [104], [105].

At any rate, antigen-antibody complexes endowed with biological activity are soluble complexes formed in moderate antigen excess, or else insoluble complexes formed at or near equivalence and later redissolved in a moderate antigen excess. As far as their capacity to produce anaphylactic-type reactions is concerned, complexes formed by co-precipitating antibody have the same activity as those formed by precipitating antibody, with the only difference that the former are also active at equivalence or even in antibody excess, although maximal activity still corresponds to complexes in moderate antigen excess [106].

The type of reaction caused by antigen-antibody complexes formed *in vitro* depends, amongst other factors, on the amount of complex injected, the route and site of injection, the duration of its action and the availability of polymorphonuclear leucocytes, necessary for an Arthus-type reaction to develop, at the site where antigen-antibody complexes get deposited [107]. It is a matter of controversy whether complexes develop their biological activity by themselves as stable units or there exists a process of dissociation of the complex followed by recombination of its components at the site and in the proportions adequate for the development of its pathogenic action [100], [108]. Possibly, in Arthus and serum sickness-type reactions the complexes act as stable units, while in some of the anaphylactic-like reactions the process of dissociation-reassociation plays a more prominent rôle.

What has been said about antigen-antibody complexes is also valid for gamma globulin aggregates produced by physical (heat) or chemical (bis-diazotized benzidine) means [109], [110], because even when the antigen may influence the localization, size, etc., of antigen-antibody complexes, their biological activity is determined by changes in the configuration of gamma globulin molecules put in apposition by their union with the antigen. This affirmation is based on the parallelism between the biolo-

gical activity of complexes or aggregates and the increase of their levorrotation, not dependent on the nature of the antigen, and an index of a change in molecular structure [111]. It should only be borne in mind that gamma globulin aggregates must act by themselves as stable units, as it is unlikely that they may dissociate and recombine again.

DELAYED-TYPE HYPERSENSITIVITY.

This is not the place for a consideration of type IV reactions according to Combs and Gell's classification, since they do not depend on antibodies, unless we consider transfer factor or any similar specific mechanism contained in sensitized cells as antibodies because of the facts that they are formed in response to an antigenic stimulus and are able specifically to react with the inducing antigen, or unless we adhere to the thesis supported by KARUSH and EISEN [112], according to which delayed-type hypersensitivity would be mediated by circulating antibody of extraordinarily high affinity present at too low concentrations to be detected by current methods [113].

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DIE HISTOPATHOLOGIE DER IMMUNITÄT UND IMMUNOPATHISCHEN REAKTIONEN

E. LETTERER.

Deutschland.

Zur Terminologie in einem Referat über die *Morphologie allergischer und immunitärer Phänomene* sei vorwegnehmend gesagt, dass wir mit einer Konzession der Begriffsauslegung an den Kliniker unter Allergie nicht die Allergie als generell erworbene Andersempfindlichkeit verstehen (v. PIRQUET) wollen, sondern von Allergie im Sinne von Hyperergie bzw. *erworbener* Hypersensitivität sprechen. Unter *Immunität* aber verstehen wir den *erworbenen vollen Schutz* des Organismus, unter welchem bei Wiederbegegnung mit dem Antigen der vorausgehenden Umstimmung keine seine Gewebe schädigende *Reaktion* einsetzt. Beschränken wir den Begriff der Immunität auf diese Fassung, so bedeutet dies, dass ein Organismus Immunität nur erwirbt gegen Antigene, die schon im ersten Kontakt in irgendeiner Form ihn schädigen, also parasitärer Natur sind und eine antiinfektiöse oder antitoxische Immunität bewirken können.

Eiweiss, Lipoid—und Polysaccharidantigene verursachen humorale Antikörperbildung, die im Blut mit dem Antigen zur Bildung von Antigen-Antikörperkomplexen (Dixon) führt. Aber weder das Antigen noch der Antikörper allein wirken zell—und gewebsschädigend, sondern erst der Komplex, der aus der Verbindung der beiden entsteht.

Immunität muss also in diesem Fall Schutz gegen den Antigen-Antikörperkomplex und seine Nachwirkungen bedeuten. Ob dies realiter der Fall ist, bleibt auch heute noch fraglich. Der antiinfektiöse und antitoxische Antikörper aber bildet mit dem Infektallergen oder dem Toxoallergen Komplexe, die zur Bindung und Zerstörung dieses Antigens führen. Insofern verhalten sich also Allergie im Sinne der Hypersensitivität und die antiparasitäre oder die antitoxische Immunität im Hinblick auf die Dynamik ihrer Wirkung entgegengesetzt.

Man kann sagen, die hypersensitivity ist eine *paradoxe Immunität,* gegen die es nur die Desensibilisierung gibt. Gegen das Bakterientoxin und die parasitär-bazillären Leibessubstanzen gibt es den die Immunität realisierenden Antikörper.

Die hypersensitivity manifestiert sich durch die *Sofortreaktion* und ist eine an ein humorales Reaktionsmilieu gebundene, auf Antikörper-

bildung beruhende *Immunopathie*. Sie tritt unmittelbar in Minuten nach der Vereinigung der Reaktionsstoffe ein und entsteht nur, wenn ein im Blut *frei* zirkulierender Antikörper, zur Bildung von Antigen-Antikörper-Komplexen zur Verfügung steht, und die Reaktion entsteht umso schneller und wird umso stärker je höher konzentriert dieser Antikörper ist. Sie ist durch Desensibilisierung zu hemmen. Ihr morphisches Reaktionssubstrat ist die Endstrombahn. Insofern ist sie eine vaskuläre bzw. eine Gewebsallergie. Die echte Immunreaktion dagegen ist die zelluläre. Sie verläuft im Milieu der *Zelle* und tritt als *delayed reaction* nach Stunden ein.

Wenngleich wir zwar von humoralen und zellulären Antikörpern und deren Reaktionen mit Recht sprechen, ist doch alles Reaktionsgeschehen letzten Endes entweder passiv oder aktiv eine zelluläre Reaktion. Die Elektronenmikroskopie hat sehr präzise Informationen über die morphischen Veränderungen an der Zelle unter dem Eindruck von Antigen- und Antikörperreaktionen erbracht.

Grundsätzlich ist zu sagen:

1. Nicht alle Reaktionen führen notwendig zu Zellschäden. Geringe Reize können auch einen anabolen Effect haben.

2. Die Wirkung der Reaktion ist zwar für eine bestimmte Antigen-Antikörperkombination spezifisch, aber das gleiche Schadensbild kann auch durch völlig andere Reize und banale Gifte erzeugt werden. Dann ist es morphisch unspezifisch und das gestaltliche Bild lässt somit keinen Schluss auf eine Immunreaktion zu. Dagegen lässt Komplementaktivierung und ihre Folge, die Lyse, auf einen Antigen-Antikörperreaktionsvorgang schliessen. Aber Lyse für sich allein ist ebenfalls unspezifisch.

3. Die Reaktionen haben zwei Verlaufsstufen: die erste ist eine *Oberflächenmembranreaktion*, die auch für die Agglutination verantwortlich ist, indem die Membran viele feine Digitationen ähnlich wie die Pseudopodien der Leukozyten ausbildet. Diese vernetzen sich an den Oberflächen und agglutinieren die Zellen miteinander. Der Vorgang verläuft ohne Komplementbindung. Die Oberflächendoppelmembranen stellen für den Antikörper eine Permeabilitätsgrenze dar, die erst mit Hilfe von aktiviertem Komplement überschritten werden kann. Dann wird die zweite Verlaufsstufe wirksam. Das aktivierte Komplement wird an den Stellen, an denen Antigen-Antikörperverbindungen haften, an die Membran gebunden und zerstört bzw. lysiert diese. Sie wird durchlässig für eintretende Antikörper, Wasser und Natrium, es kommt zur Zellschwellung und zum Austritt von Ribonukleotiden und Kalium. Der in die Zelle eingedrungene Antikörper wirkt mit Komplement in der gleichen Form an den Membranen der Lysosomen und Mitochondrien und des Kerns. Wiederum tritt Schwellung und Lyse ein. Die Enzyme innerhalb der Lysosomen werden aktiviert und zerstören die Reststruk-

turen der Zelle. Ein nicht zu weit vorgeschrittener Zellschaden ist unter Umständen wiederherstellbar. Haptene und Reagine, Antikörper von zytotoxischem Charakter, das heisst gegen bestimmte Zytoplasmaantigene, haben alle den gleichen Effekt. Das geschilderte Reaktionsbild entspricht vorgangsmässig der Sofortreaktion. Jedoch ist dieser Name nicht an der Zelle, sondern am Gewebe geprägt, weil das, was sofort reagiert, die Endstrombahn mit ihren Kapillaren ist, sobald sich ein Antigen-Antikörperkomplex gebildet hat. Der sofortige Eintritt der Reaktion wurde bestimmend für die Signatur. Diese Reaktion ist wie gesagt präventiv zu desensibilisieren. Aber in diesem Falle wird der Antikörper und nicht das Antigen neutralisiert, daher "paradoxe Immunität".

4. An den Zellen entstehen auch bei der Spätreaktion die *gleichen* Bilder. Grundsätzliche Unterschiede in der Morphe sind nicht vorhanden. Die Spätreaktion erscheint nur deshalb verzögert, weil die Zellen, die in die Reaktion eintreten, in grösserer Zahl erst aus den schon vorhandenen sensibilisierten Zellen gebildet werden, wenn dar Antigen erneut in das Gewebe eintrat. Sie bilden sich aus den von GELL geschilderten sogenannten "islands", den perivaskulären Zellinseln. Sie sind lymphohistiozytärer Natur, aber nach einigen Tagen treten in diesen Inseln auch einige Plasmazellen auf. Da Plasmazellen nur humorale Antikörper bilden, ist bei dieser Reaktion nicht ganz klar und eindeutig zu trennen zwischen humoraler Schnell—und zellulärer Spätreaktion. Die Zellen enthalten an ihrer Oberfläche und im Zytoplasma einen Stoff, der im Anschluss an die erste Begegnung mit dem entsprechenden Antigen gebildet, aber nicht in die Säfte abgegeben wurde, daher zellständig genannt wird und dem manche Antikörpernatur zusprechen, andere aber auch nicht. Auf der Existenz dieses Stoffes beruht z. B. bei der Tuberkulose die Lymphozytolyse. Sie verläuft nur mit Komplementbeteiligung und die Oberflächenphänomene, die lytischen Zerstörungen der Membranen und Zellorganellen sind genau die gleichen. Das kann aber nicht bedeuten, dass zwischen Sofortreaktion und Spätreaktion vielleicht kein oder nur ein quantitativer Unterschild sei.

5. Von grundsätzlich gleichem Typ der zellulären Immunität, aber in gewissen Hinsichten abgewandelt, ist die *Transplantationsimmunität.* Das Wesentliche dieser zellulären Reaktion ist, dass bei ihr nicht Säfte ("humores") oder Fraktionen von solchen (Globuline) in die Reaktion eingreifen, sondern einerseits Zellen die Rolle des Antigens bzw. Antigenträgers und ebenfalls Zellen die Rolle des Antikörpers bzw. Antikörperträgers spielen. Wenn eine solche doppelt zelluläre Reaktion eintritt, wie dies bei der Kontaktallergie, der Transplantationsreaktion und auch bei der autoallergischen Encephalomyelitis der Fall ist, dann kommt es zur Neubildung von Zellen mono- und histiozytärer Natur. Diese neugebildeten Zellen dringen in das antigenhaltige Zellgewebsmilieu ein und die Zellen des Transplantates, welche die Rolle des Antigens spielen, werden von den in das Reaktionsfeld eingewanderten Mesen-

chymzellen, welche die Rolle des Antikörpers spielen, umlagert. Infolge dieser immunitären Kontaktierung entstehen in den antigenischen Zellen Vakuolen. Histiozyten oder Lymphozyten dringen in die Antigen-Zellen und in die Vakuolen ein und unter lytischen Symptomen geht die Zelle zugrunde. Die ersten elektronenoptischen Darstellungen entsprechen nicht in allem den lichtmikroskopischen Befunden.

Entsprechende Beobachtungen sind auch in vitro möglich (ROSENAU und KOPROWSKI). Makrophagen, die aus Tieren stammen, welche mit Kulturfibrozyten sensibilisiert worden waren, können in einer Subkultur der gleichen Fibrozyten, wie wie zur Impfung verwendet wurden, nun mit diesen kontaktil agglutinieren und sie zum Schwund bringen (*contactual agglutination*). Diese Art Zytolyse bedarf keines Komplementes, während die zytotoxischen Antisera zur Zerstörung von Zellen Komplement benötigen. Bei dem genannten Anlagerungsprozess der contactual agglutination kommt es zur Ausbildung von Zellfortsätzen zwischen der antigenischen Zelle und der Antikörperzelle und unter einer sogenannten "Austausch-Pinozytose" erfolgt das Absterben der antigenischen Zellen auf dem Wege der vakuolären Degeneration. Im Sinne des klassischen Immunitätsbegriffes wäre dies die Manifestation einer echten Immunität, bei welcher durch ein entsprechend ausgebildetes Antikörperprinzip das Antigen paralysiert oder zerstört wird.

6. Wenden wir einen kurzen Blick nun von den Zellen auf die Gewebe. Die Reaktion der Gewebe wird in ihrer Morphe vor allem dadurch charakteristisch modifiziert, dass das Reaktionssubstrat insgesamt nicht von der Zelle allein, sondern vom gesamten *Histion* dargestellt wird. In diesem ist das Gefäss als *Endstrombahn* mit Arteriole und Kapillare der führende Reaktionsträger. In der Endstrombahn ist die ausschlaggebende Struktur die glatte Muskelzelle der Arteriole und die muskuläre Kapillarpförtnerzelle an den Kapillarbahnverzweigungen. Ihre Kontraktion verursacht die Zirkulationsänderungen in der Strombahn. Der zirkulierende Antikörper aber bildet bei erneutem Kontakt mit dem Antigen einen *Antigen-Antikörperkomplex*. Dieser Bildungsvergang führt über die Wirkung von Mediatorstoffen zur Kontraktion der glatten Muskulatur und zur Steigerung der *Permeabilität* der Kapillarwand. Der Antikörper selbst erzeugt mit seiner Reaktion zusätzlich Konglutination von Leukozyten und Plättchen. Dadurch entstehen Mikrothromben, die wiederum zirkulationsbehindernd wirken. Die Permeabilitätssteigerung aber lässt Antigen-Antikörperkomplexe durch die Gefässwand treten, erzeugt dort Ödem, Blutung und fibrinoide Degeneration. Den ganzen sehr komplexen Vorgang, der integrierend an zirkulierende humorale Antikörper gebunden ist, bezeichnen wir als *Arthus-Phänomen*.

Bei der verzögerten Reaktion gibt es keinen freien Antikörper im Blut. Das Antigen reagiert erst in Stunden oder Tagen mit neuen, aus dem Reaktionsherd proliferierten Histiozyten und Monozyten. Die neugebildete Mesenchymzelle selbst reagiert mit dem neueingebrachten Antigen in der oben schon gezeigten Weise wie bei der Immunlymphozytolyse.

7. Während nun der Morphologie der Hypersensitivität seit zwei Menschenaltern die intensive Aufmerksamkeit der Forscher gewidmet war, ist die Frage, ob es eine charakteristische *Morphologie* der *erworbenen Immunität* gibt, offenbar nicht so attraktiv.

Da die Forschung in nicht geringem Ausmass auch von praktisch-therapeutischen Gesichtspunkten dirigiert wird, war in einem Zeitalter der Sulfonamide und Penicilline mit einem viel besser protektiven Effekt gegen bazilläre Infekte als ihn die Immunsera haben, das Interesse für eine vertiefte Kenntnis der Morphologie des Zustandes erworbener Immunität nicht mehr gross.

Wir wissen wenig über die Mechanismen, welche die erworbene Immunität bei bazillären und viralen Infekten bedingen. So ist es z. B. bis heute nicht klar, auf welche Weise und wo ein gegenüber Typhus vakzinierter Mensch eine neue Infestation mit Typhuserregern paralysiert, zellulär oder humoral oder mit beiden Mechanismen? Wir wissen, dass die Vakzination protektiv sehr gut wirkt, aber wis wissen nur teilweise, wie sie wirkt. Cruikshank nennt diese Wirkung ein "Rätsel".

Sensibilisierte Lymphozyten lassen, wie EHRICH und später NOSSAL gezeigt haben, eine Immunadhärenz gegenüber dem Erreger erkennen; ob das zur Vernichtung des Erregers führt wissen wir nicht. Agglutinine klumpen die Erreger zusammen. Histologisch können sie als Agglutinate im Blut und in den Sternzellen der Leber gefunden werden. Unter Komplementzutritt können Bakterienagglutinate der Lyse ebenso verfallen wie agglutinierte Zellen, und der Schluss liegt nahe, dass die oben für Blut- und Gewebezellen beschriebenen Phänomene auch an der Membranoberfläche der Bakterienzelle und dann im Innern derselben sich abspielen. Aber die Morphe der vollendeten Immunität hat kein spezifisches Gesicht und ist deshalb umso schwerer zu analysieren; sie kann vom völlig negativen Zell- und Organbefund bis zu definierten Manifestationen reichen. Unter immunitärem Schutz können sich Antigen und Antikörper reagierend oder reaktionslos begegnen. Dabei herrscht eine gewisse Gegensätzlichkeit, denn je stärker die Wirkung der Immun-Antikörper ist, desto geringer ist die Beteiligung morphischer Erscheinungen. Dei einer der vollendeten Immunität nur angenäherten Reaktionslage, der sog. infinitesimalen Immunität, bei welcher exogene oder endogene Gründe die volle Immunität nicht erreichen lassen, ist die zelluläre Reaktion besonders stark. Hyperplasie von Mesenchymzellen, Mitosen, Riesenzellen, verstärkte Phagozytose, perivaskuläre Histiozytenproliferationen in Lunge, Leber, Niere, Herz sind die morphischen Merkmale solcher Zustände, die wir bei Typhus, Rheumainfekten, chronischer Sepsis, aber auch in bestimmten Stadien der Tuberkulose finden. Dabei zeigt sich, dass den Monozyten in vivo offenbar die grössere Bedeutung für den Effekt eines immunitären Schutzes zukommt als der phagozytären Tätigkeit der Leukozyten. Von der antitoxischen Immunität abgesehen, bei der ein humorales Antitoxin die Giftbindung bewirkt, ist sowohl die antibakterielle wie die antivirale Immunität als eine zelluläre Immunität anzusehen.

THE KININ SYSTEM IN ANAPHYLAXIS AND ALLERGY *

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Twenty five years ago, if one was asked about the mechanism of anaphylaxis, the only possible answer was that it resulted from "unspecific damage" inflicted by the antigen upon the cell membrane. As a consequence of this unspecific damage to the cell membrane, many substances, among which histamine was by far the most important, would diffuse out of the cell where they were kept normally in a bound form.

To call upon an enzymatic mechanism to explain such a release of active materials was likely to arise strong criticism. More than that, any mention of a participation of blood or enzymes derived from the blood or interstitial fluids was considered a heresy and the heretic to be purified by eternal flames.

Nowadays, the situation has been more than reversed. I would dare say that the heretics are those who do not believe in a complicate enzymatic process underlying the mechanism of anaphylaxis and allergy. The release of histamine appears to involve pathways of the carbohydrate metabolism, formation of high energy compounds (probably ATP) as a prelude to the breakdown of the membrane of mast cells. Parallel to this process, activation of proteases takes place to release polypeptides such as bradykinin, kallidin and so forth.

Anaphylactoid reactions of venoms and enzymes. In the beginning, since histamine was thought to be the only mediator of the anaphylactic reaction, the attention was focused on finding an enzyme able to release it from its loose combination with the cell constituents. In the latest thirties, a great impetus was given in that direction by the experiments of FELDBERG and KELLAWAY (1937-38) showing that the venoms of certain snakes, such as the *Naja naja* and *Denisonia superba,* were able to deplete the perfused guinea pig lungs of their histamine stores. Since some of these venoms were strongly proteolytic, we have tried (ROCHA E SILVA, 1939-40) a pure enzyme, crystalline trypsin, to see if it might reproduce some of the features of anaphylaxis and of the action of the

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snake venoms. We have found that upon the plain muscle of the uterus and intestine of many species, and also upon the blood pressure of the cat, the rabbit and the dog, trypsin faithfully reproduces the effects of the venoms, as shown by FELDBERG and KELLAWAY, and these effects showed the phenomenon of tachyphylaxis so characteristic of the action of venoms and of "in vitro" and "in vivo" anaphylaxis. On the isolated ileum of the guinea pig, trypsin when added in small concentrations produced a slowly developing tonus which resisted successive washings. Repeated additions of the same dose of trypsin produced a state of tachyphylaxis very similar to that described by FELDBERG and KELLAWAY with the snake venoms. When injected intra-arterially, trypsin produced in the dog, the cat and the rabbit, shock-like conditions (trypsin shock) almost indistinguishable from that produced by the antigen in the sensitized animal (ROCHA E SILVA, 1940-41). When perfused through the isolated guinea pig lung, trysin released small but detectable amounts of histamine. These experiments have been confirmed by many workers (TRETHEWIE and DAY, 1949). Later on, in collaboration with Dragstedt (DRAGSTEDT and ROCHA E SILVA, 1941; DRAGSTEDT, WELLS and ROCHA E SILVA, 1942) we showed that trypsin releases histamine from the white blood cells (platelets) of the rabbit. This effect of trypsin was again confirmed by MCINTIRE et al. (1950), MCINTIRE (1956) and HUMPHREY and JAQUES (1955). Papain by its component which digests benzoyl-L-argininamide, was found to release histamine from rabbit's white blood cells (ROCHA E SILVA and ANDRADE, 1943).

If we go back to the latest thirties, we have to notice that some of the venoms utilized by FELDBERG and KELLAWAY (1937-38) and some of the most potent such as that of the *Naja* and of the *Denisonia superba*, are predominantly hemolytic, because of the presence of a powerful phospholipase (lecithinase) which would transform lecithin into lysolecithin, by splitting off one molecule of oleic acid. Lysolecithin was found by the Australian workers to release histamine and produce some of the pharmacological effects of the snake venoms.

As the cell membrane is usually assumed to be a mosaic of lipoproteins the damage inflicted upon it by histamine releasers could be explained either by a direct lytic effect of proteolytic enzymes upon the protein part of the membrane or by a lipolytic process under the action of lysolecithin. These two possibilities were postulated by FELDBERG (1941) in a review on the subject. The lipolytic theory was revived recently by Uvnäs (1958) on the basis that the most powerful damaging agent upon the mast cell membrane was the phospholipase from snake and bee venoms. This conception could not overcome the obvious objection that the release of histamine is not necessarily accompanied by a hemolytic process. Furthermore, the hemolysis produced by complement is more likely to depend upon esterolytic enzymes (proteases) than on a phospholipase A, which to my knowledge has never been convincingly isolated from the mammalian body.

In contrast to indirect doubtful evidences of participation of lipolytic enzymes in the genesis of anaphylactic and allergic symptoms, we

have to recall the large body of evidences to show activation of proteases and proteolytic enzymes in the course of these reactions (ROCHA E SILVA et al., 1946; ROCHA E SILVA, 1950; UNGAR and HAYASHI, 1958). In the rat, participation of a chymotrypsin-like enzyme has been recently postulated to explain rupture of mast cells by the antigen (AUSTIN and BROCKLEHURST, 1960-61; KELLER, 1962). If we sum up all the physiological actions of proteolytic enzymes as have been demonstrated with trypsin it could be made a point to stress that participation of such enzymes in anaphylaxis and allergy is probably of a more fundamental significance than a simple release of histamine. This will become clear when we analyse the participation of the kinin system.

Participation of mast cells. With the discovery that histamine is to a great extent stored in mast cells (RILEY and WEST, 1952; MOTA et al. 1953), the problem of its release shifted from the outside to the inside of these cells, and any lytic enzyme that might disrupt such reservoirs of histamine would be found inside rather than outside the boundaries of such cells. Along such lines there are indications that exo- and endopeptidases (aminopeptidase, chymotrypsin or trypsin-like enzymes) have been identified in the mast cells (BENDITT and ARASE, 1959; LAGUNOFF and BENDITT, 1963).

The problem has been complicated by the observation that many basic compounds, such as simple amines, diamines, diamidines and diguanidines or alkaloids, such as D. tubocurarine, atropine, strychnine, and so forth, release histamine (ALAM et al., 1939; SCHILD and GREGORY, 1947; ROCHA E SILVA and SCHILD, 1949; MAC INTOSH and PATON, 1949; PATON, 1951) apparently by rupturing mast cells in the tissues of the rat and cat skin, but are almost inactive as histamine releasers from the rabbit's platelets or the guinea pig lung. Especially upon the guinea pig lung, the doses of the most powerful compound (48/80) must be increased to values at least 100 times the concentrations which are active upon the rat's mast cells. As the lung of the guinea pig is the most sensitive structure to show release of histamine in anaphylaxis, it is altogether unlikely that the antigen acts in the guinea pig through the intermediary action of basic compounds.

Histamine release by anaphylatoxin. Another finding that might throw light on the mechanism of histamine release in guinea pig anaphylaxis was the observation that the serum of the guinea pig or of the rat, when incubated with polysaccharides such as agar, dextran or starch, or with the complex antigen-antibody, generates a principle which releases large amounts of histamine from the perfused lung of the guinea pig. This principle, probably identical with the old anaphylatoxin or serotoxin of FRIEDEMANN (1909), FRIEDBERGER (1909) and BORDET (1913), is generated through an enzymatic process from the protein fractions of normal plasma. As regards the lung of the guinea pig, anaphylatoxin is the most potent histamine releaser ever found in the mammalian body, and its toxic effect on this species can be com-

pletely explained by its capacity of releasing histamine (ROCHA E SILVA, BIER and ARONSON, 1951; ROCHA E SILVA and ARONSON, 1952). It reproduces by itself the "in vivo" and "in vitro" effects of anaphylaxis in the guinea pig, contracts the guinea pig ileum, with tachyphylaxis, and this effect is totally blocked by anti-histaminics (ROCHA E SILVA, 1952; ROCHA E SILVA, 1954; ROTHSCHILD and ROCHA E SILVA, 1954). The alterations of mast cells in the guinea pig lung produced by anaphylatoxin are indistinguishable from those produced by anaphylaxis in this animal species (MOTA, 1959). Anaphylatoxin also releases histamine from the rabbit's blood cells and this effect is blocked by salicylates and other inhibitors of the anaphylactic release (HAINING, 1956; ROTHSCHILD, 1961).

Mechanism of histamine release. The release of histamine is a complicated process not yet completely clarified. We know now that the system involved is probably a multiple-enzyme process, to which some steps of the carbohydrate metabolism are not alien. Temperature and pH optimum are important factors in the mechanism by which histamine is released from stores in mast cells or in rabbit's platelets. No matter which agent is utilized, anaphylaxis, anaphylatoxin or basic compounds (as 48/80), there appears to be some commom mechanism underlying the release of histamine from different cell structures. If the temperature is lowered to 2°C only traces of histamine are released. The optimum of pH appears to be the same for all three types of releasing agents. Some species differences should be mentioned. Though 48/80 acts optimally upon the rat's mast cells, it is extremely inefficient as a histamine releaser from the guinea pig lung and does not act at all upon the rabbit's platelets; contrariwise, anaphylatoxin, which is a very potent releaser of histamine from the guinea pig lungs, is almost inert upon the rat's mast cells. Nevertheless, if we consider the mechanism by which anaphylaxis and anaphylatoxin release histamine from the guinea pig lung, and the one by which 48/80 releases histamine from rat's mast cells, a striking similarity occurs, indicating the existence of some mechanism by which histamine is set free in all circumstances. Also, the mechanism by which histamine is set free from rabbit platelets bears analogies with the release from mast cells by 48/80 or anaphylatoxin.

The use of enzymatic inhibitors gave interesting results. It was shown by MOUSSATCHÉ and DANON (1958) that inhibitors of oxidative phosphorylation such as 2,4-dinitrophenol (DNP) and other phenol derivatives block the histamine release from slices of the sensitized guinea pig lung brought into contact with the antigen. This has been confirmed and extended to the release of histamine by basic compounds as 48/80 (HÖGBER and UVNÄS, 1957; UVNÄS, 1958; ROTHSCHILD, VUGMAN and ROCHA E SILVA, 1961) acting upon histamine rich structures, as the rat's mesentery or diaphragm. It was already known that iodoacetate (MONGAR and SCHILD, 1955; MOUSSATCHÉ and PROUVOST-DANON, 1958), anoxia (PARROT, 1942; MONGAR and SCHILD, 1955) and cyanide (PARROT and RICHET, 1944; MONGAR and SCHILD, 1955) prevent the release of histamine in anaphylaxis. These inhibitors were also found to be effective to

prevent the release of histamine by basic compounds as shown by MOTA and ISHII (1960), ROTHSCHILD, VUGMAN and ROCHA E SILVA (1961) and also by UVNÄS (1958) and HÖGBER and UVNÄS (1960). A very important verification along these lines was the fact observed simultaneously by several investigators, that glucose prevents the inhibitory effect of such agents like DNP and congeners, anoxia and cyanide, though iodoacetate and parachlormercuribenzoate are not affected by the presence of an excess of glucose in the medium. Table I shows the effect on the release of histamine by 48/80 (on the rat diaphragm) of metabolic inhibitors known to act at different sites in the enzymatic system. These inhibitors can be roughly divided into two groups: those whose action is dependent on the presence of glucose (DNP, cyanide and anoxia) and those which continue to act inhibiting the release in spite of the presence of glucose (iodoacetate, parachlormercuribenzoate). Phlorhizin has a position of its own: it only inhibits the release of histamine if added together with DNP. Addition of an excess of glucose will not revert the inhibition in presence of phlorhizin (ROTHSCHILD, 1961). From these and other experiments (DIAMANT, 1960; PROUVOST-DANON and MOUSSATCHÉ, 1961; ROTHSCHILD, VUGMAN and ROCHA E SILVA, 1961 and others) there is now an increasing body of evidence indicating that production of ATP by the glycolytic cycle of the carbohydrate metabolism is an important step in the mechanism of histamine release. When associated with histamine in rabbit platelets and rat mast cells, serotonin seems to be released by a similar mechanism, though its participation in the mechanism of shock in the guinea pig and the rabbit is doubtful. It seems that serotonin might play a more definite role in anaphylaxis in rodents, especially in mice (GERSHON and ROSS, 1962). We have, however, to postulate

TABLE I

Effect of metabolic inhibitors on the release of histamine from the isolated rat diaphragm by compound 48/80; influence of glucose on the rate of inhibition (according to Rothschild, 1961).

INHIBITOR	% Inhibition	
	No Glucose	Glucose 0.1 %
Dinitrophenol, 0.5 mM.	88	3
Anoxia (N, atmosphere)	63	19
Sodium cyanide, 1 mM.	95	20
Thiopental, 1 mM.	65	22
Sodium salicilate, 25 mM.	95	33
Sodium salicylate, 50 mM.	100	98
p-Chloromercuribenzoate, 0.5 mM.	95	84
Iodoacetate, 3 mM.	100	100
Sodium fluoride, 40 mM.	82	78
Phlorhizin, 2.2 mM.	5	35
Phlorhizin + DNP	100	88

a second phase of the phenomenon, assuming that a final step would be the activation of a lytic enzyme which might break down the structure of the mast cells, as described above. The scheme presented in fig. 1 gives an approximate idea of the mechanism of histamine release according to our present view of the phenomenon.

Participation of other agents in anaphylaxis. Now, I would like to face the problem of the mechanism of anaphylaxis and allergy from its proper angle, without identifying it with the mechanism of histamine release. After the discovery of the highly potent and specific anti-histaminics, the importance of histamine as a mediator in such reactions has been confined to its true domain. We know that there are anaphylactic reactions which are easily prevented by anti-histaminics, even by the weakest ones, as those first described by STAUB (1939) and that there are other reactions or symptoms which are not blocked by even the most potent ones, such as neo-antergan or phenergan. I give for instance the example of the anaphylactic shock in the guinea pig. Though it is known that the acute death by broncho-constriction can be fairly well abolished by all anti-histaminics, the so-called protracted shock is not prevented by anti-histaminics. This has been demonstrated recently by HAHN et al. (1961) who have shown that anti-histaminics though protecting guinea pigs from histamine shock or from acute death by anaphylactic shock, do not prevent the death of a large proportion of animals later on, with abdominal herorrhages or hyperemia and collapse after prolonged prostration. It was also shown that anaphylatoxin shock in guinea pigs is more like histamine shock than anaphylactic shock, the constriction of the bronchioli produced by anaphylatoxin being completely prevented by such doses of anti-histaminics which prevent a similar effect produced by histamine. The experiments by HAHN et al. (1961) definitely indicated that other substances, besides histamine, would play a role in guinea pig anaphylaxis. This, of course, was already suspected by many workers and I remind the interesting experiment by SCHILD (1949) showing that an uterine horn of a sensitized guinea pig, made refractory to histamine by addition of an excess of it to the bath, still reacted strongly to the addition of the antigen. That anti-histaminics could not prevent totally the "in vitro" anaphylactic reaction of stripes of the guinea pig ileum was also demonstrated by HAWKINS and ROSA (1956). These difficulties led DALE (1950) to the assumption that part of the histamine which plays a role in anaphylaxis is so intimately released inside the reacting structures that even the most powerful anti-histaminics would have no access to its place of action. This idea of "intrinsic histamine" lost most of its grounds when it was demonstrated that the histamine which acts upon the plain muscle structures of the guinea pig bronchioli and also of the intestinal walls, comes from mast cells in the connective tissue and is therefore, "extrinsic" to the reacting structures. Furthermore, the part of the reaction elicited by anaphylaxis, and which is not prevented by anti-histaminics, is of a different kind, that is of a slow type, like that produced by snake venoms, trypsin or

the now available slowly reacting substances, such as bradykinin and kallidin (l. lysil-bradykinin).

At least three kinds of slowly reacting substances have been postulated to play a role in anaphylaxis:

a) The SRS released by snake venoms in the experiments by FELDBERG and KELLAWAY (1937-38); this substance was restudied recently by VOGT (1956) who demonstrated that it has the nature of an acidic material quite distinct from bradykinin and other polypeptides;

b) The SRS-A released from a sensitized guinea pig lung by perfusion with the antigen; this material was first found by KELLAWAY and TREFFREWIS (1940) and re-studied more extensively by BROCKLEHURST (1956-60). Apparently it is not a polypeptide and might be identical or very similar to the principle studied by CHAKRAVARTY (1960) with the name of SRS; this material arises from mast cells when disrupted by basic releasers or by anaphylaxis; very little is known about its composition, besides the indication that it should also be acidic;

c) The bradykinin-like material, of a polypeptidic nature which has been shown to be released in anaphylactic and peptone shock in the dog (BERALDO, 1950) and found in the blood of guinea pigs submitted to anaphylactic shock (LAHIRI and BROCKLEHURST, 1961).

Release of kinins. We are going to confine our discussion to the substances of the third group (c) to which we might give the general name of kinins. In a recent review on the mechanism of allergic reactions, FELDBERG (1961) says: "The reasons why these peptides (kinins) should be considered ideal mediators of the anaphylactic and allergic reactions are of three categories:

1. Their high activity;
2. The fact that their action upon plain muscle and the circulation are resistant to anti-histaminics and also to inhibitors of 5-HT;
3. The easiness of their formation in the body by the action of enzymes which can be activated by kinases from blood and tissues, as well as by simple physical alterations, as for instance, when plasma comes into contact with glass or by dilution."

To these reasons one might add a fourth one that might explain the failure to detect active bradykinin in the circulating blood in the case of its release: the rapidity of its destruction by an enzyme present in plasma or in organs, such as the kidney. It has been recently claimed that bradykinin is destroyed by an enzyme in plasma showing the specificity of carboxypeptidase-B, releasing the terminal arginine in the sequence

Arg — Pro — Pro — Gly — Phe — Ser — Pro — Phe — Arg
↑
carboxypeptidase-B

Many inhibitors of carboxypeptidase B were found to protect destruction of bradykinin in blood (ERDÖS, 1961-62). We have found that some substances containing sulfhydryl groups, such as BAL, thiogly-

TABLE II

Pharmacological actions of bradykinin.

Effect	Test object	Effective dose
Smooth muscle contraction	Isolated guinea pig lung	1 ng/ml
	Isolated rat uterus	0.1 ng/ml
	Rat stomach (Vane prep.)	5-10 ng/ml
Smooth muscle relaxation	Isolated rat duodenum	0.8-1.2 ng/ml
Constriction of bronchioles	Guinea pig (i. v.)	0.2-0.5 μg/kg
	Cat (i. v.)	12.8 μg/kg
Vasodilatation	Cat skin	0.1 μg
	Human skin (i. a.)	0.1-1.0 μg
	Man (i. a.)	0.4-2.0 μg
	Pia (guinea pig, i. v.)	1.0 μg/kg
	Cerebral (dog, i. v.)	0.5-3.0 μg/kg
	Coronary (G. pig, isol. heart)	1.0 ng/ml
	Coronary (Cat, isol. heart)	10.0 ng/ml
Lowered blood pressure	Cat (i. v.)	0.4-0.5 μg/kg
	Rat (i. v.)	1.5 μg/kg
	Guinea pig (i. v.)	0.2 μg/kg
	Dog (i. c.)	0.5 μg/kg
	Cock (i. v.)	74.0 μg/kg
	Man (i. a.)	0.03-0.06 μg/kg
Increased capillary permability.	Guinea pig (i. d.)	0.1-5 ng
	Rabbit (i. d.)	0.1-1.5 ng
Edema	Rat paw (s. c.)	1-100 μg
Pain production	Human blister base	0.1-1.0 μg/ml
Antidiuresis	Dog (unanesth., i. c.)	0.5-1.0 μg/kg

i. v. = intravenous; i. a. = intra-arterial; i. c. = intracarotideal; i. d. = intradermal; s. c. = subcutaneous.

colic acid, and also chelating agents such as 3-hydroxyquinoleine, are able to protect bradykinin from its destruction by the plasma enzyme (FERREIRA and ROCHA E SILVA, 1962). It was already known that cysteine protects bradykinin (PICARELLI et al., 1962). Some of these compounds, especially BAL, are able to potentiate the pharmacological actions of bradykinin (CORRADO, FERREIRA and ROTHSCHILD, in the press).

A great impetus to the study of the physiological actions of bradykinin was given by its synthesis by BOISSONNAS et al. (1960). Table II gives a summary of the physiological actions of bradykinin, with the threshold doses in micrograms or nanograms of the pure or synthetic material. Some of its effects are strongly suggestive of a participation in phenomena involving smoth muscle stimulation, increased capillary

permeability, edema formation, vaso-dilatation and increased local blood flow. Bradykinin and its derivative kallidin (l. lysil-bradykinin) were found to be powerful bronchiolar constrictors in the guinea pig lung (COLLIER, 1961) and their spasmogenic effect could be counteracted by antipyretic drugs, such as acetylsalicylate, pyramidon, etc. Upon the capillary bed in most species of animals, bradykinin was found to be the most potent agent, being about 20 to 200 times more active than histamine itself. Though not eliciting a typical triple response in the human skin (HERXHEIMER and SCHACHTER, 1959) bradykinin produces a local flush, and when injected intra-arterially, it elicits a strong vaso-dilating effect, with flush of the face in doses comparable or smaller than those of histamine needed under similar circumstances (FOX et al., 1961; EHRINGER et al., 1961). Of great importance from a clinical point of view is the recent finding by OATES et al. (1964) that the flush of the face in individuals with liver carcinoid receiving an injection of adrenaline, should be ascribed to a release of bradykinin, rather than of serotonin, as previously believed.

Changes in bradykininogen content during anaphylactic shock. The evidences of participation of bradykinin in anaphylaxis are mainly derived from the observation of a decrease of the stores of bradykininogen in plasma. As it is difficult to detect free active bradykinin in the blood, because it is rapidly destroyed or captured by the tissues, it was thought more rasonable to have an indirect measure of its release, by estimation of the stores of bradykininogen before and after the onset of the shock. A micromethod of estimation of bradykinin was developed in our laboratory (DINIZ et al., 1962) and has been used since with success to explore the possibility of participation of bradykinin in many physiological and pathological processes. The amounts of bradykininogen to be found in plasma of humans and of several species is presented in Table III. A pronounced fall in bradykininogen content was detected in the rabbit and the dog (DINIZ and CARVALHO, 1963; DINIZ, CARVALHO and CORRADO, 1963)

TABLE III

Bradykinin content in plasma (ug/ml)

	Average	Range
Man	10.6	7.4 - 14.3
Ox	14.5	11.9 - 17.0
Horse		8.8 - 9.9
Sheep	9.5	6 0 - 6.9
Pig	6.4	2.8 - 5.8
Dog	4.1	4.5 - 6.5
Rabbit	5.5	8.8 - 11.4
Guinea Pig	10.1	7.9 - 10.0
Rat	8.9	1.3 - 2.5
	2.0	

after severe shocks produced by trypsin, kallikrein and nagarse (a proteolytic enzyme from *B. subtilis*) with a concomitant increase of extractable free bradykinin in the circulating blood. With variants of this technique, or with simpler techniques (as utilizing saliva, instead of trypsin, for instance) a decrease in circulating bradykininogen in rabbits submitted to anaphylaxis (LAMBERT et al., 1964) or of guinea pigs under similar conditions have been described (BROCKLEHURST, personal communication).

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ASPECTS BIOLOGIQUES DE LA TOLÉRANCE IMMUNITAIRE

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Sensibilisation et tolérance sont les deux formes de réaction immune par lesquelles un Mammifère répond à un stimulus antigénique.

Avant d'aller plus loin, il convient de préciser la signification de ces deux termes, que je serai amené à employer, ici, fréquemment.

Les termes "sensibilisation", "immunité", "immunogène" sont synonymes. L'immunité, comme la sensibilisation, se traduit par una réponse immunologique classique, comprenant soit la synthèse d'anticorps circulants, soit la formation de cellules spécifiquement sensibilisées, responsables de l'hypersensibilité retardée.

"Tolérance" ou "paralysie immunitaire" se définissent par l'inhibition de l'immunité.

Un Mammifère normal peut réagir à l'antigène soit par l'immunité, soit par la tolérance. Cela dépendra, ainsi que nous le verrons ultérieurement, d'une foule de facteurs, y compris la quantité ou la nature de l'antigène, l'âge de l'animal, la parenté génétique entre receveur et donneur, la différence dans leur degré d'histocompatibilité.

Ce qui découle de cet exposé c'est que "immunité" et "tolérance" sont deux aspects du même phénomène. De plus, les travaux consacrés à cette question, au cours de ces dernières années, permettent d'avancer qu'"immunité" et "tolérance" ne sont pas deux phénomènes opposés ni même qualitativement différents, mais des variantes quantitatives du même type de réponse immunologique.

Tolérance induite à l'aide de cellules vivantes.

Cette forme de tolérance immunitaire a été décrite par BILLINGHAM, BRENT et MEDAWAR [1] en 1953: Lorsque les cellules spléniques d'une souris adulte sont administrées à un foetus d'une souris allogénique par rapport au donneur, l'animal ainsi traité devient tolérant, à l'âge adulte, à l'égard des cellules et des tissus provenant du donneur ou de ses congénères isogéniques. Le même phénomène peut s'observer spontanément, dans la nature, chez les jumeaux dizygotes dans le cas d'anastomoses synchoriales [2], ou expérimentalement par parabiose des membranes allanto choroïdiennes des oeufs embryonnés [3]. La tolérance immunitaire

ainsi induite au cours de la vie embryonnaire ou périnatale a été interprétée comme le reflet de l'incapacité de l'organisme foetal de répondre aux substances antigéniques que contiennent les cellules étrangères. Nous verrons que l'opinion, à cet égard, a sensiblement évolué depuis.

L'induction de l'état de tolérance n'est pas l'apanage de toutes les cellules vivantes. Les travaux de ces dernières années ont mis en évidence que, seules, les cellules nucléées d'origine mésenchymateuse, à l'exclusion des cellules épithéliales, musculaires, et, bien entendu, des hématies non nucléées, possèdent cette propriété remarquable. Dans la famille même des cellules mesenchymateuses, il existe, à cet égard, des différences, et on s'accorde à reconnaître au lymphocyte un rôle privilégié. La rate et les ganglions lymphatiques étant les réservoirs normaux de ces cellules, c'est dans ces organes que l'immunologiste va les cueillir pour les études expérimentales.

La nature intime des mécanismes par lesquels les cellules lymphocytaires induisent la tolérance immunitaire n'est pas encore connue. On s'accorde, en général, à admettre que cet état est lié à la capacité de ces cellules de coloniser l'organisme d'emprunt et à y maintenir, en permanence, une certaine quantité d'antigènes tissulaires du donneur. Cette notion de la nécessité de la présence de l'antigène, pour perpétuer un état de tolérance, est une acquisition très importante et nous y reviendrons ultérieurement.

Autres formes de tolérance

La faculté d'induire la tolérance n'est pas limitée aux cellules nucléées vivantes, comme on l'a pensé pendant un certain temps. Selon les résultats récemment obtenus, un état de tolérance similaire, avec survie d'homogreffe, peut être obtenu en administrant, à des animaux, des extraits acellulaires. Ainsi, MEDAWAR [4] a montré que l'administration d'un extrait splénique permet d'obtenir une prolongation très significative de l'homogreffe de peau chez des animaux allogéniques adultes.

Des résultats similaires ont été enregistrés par MARTÍNEZ et collaborateurs [5]. On peut rapprocher, dans une certaine mesure, ces observations de celles faites avec des antigènes solubles. Ainsi, dès 1942, FELTON et OTTINGER [6] ont montré que l'injection, à la souris adulte, de polyoside de Pneumocoque détermine un état d'irresponsivité immunologique à l'égard de cet antigène, qui peut persister toute la vie. Ces auteurs ont dénommé ce phénomène "paralysie immunitaire". L'observation de FELTON et OTTINGER est restée longtemps incomprise et a été considérée comme un fait biologique exceptionnel.

Au cours de cette dernière décade, toute une série de travaux, dont ceux de HANAU et OYANA [7], de DIXON et MAURER [8], de DUBERT et PARAF [9], de SMITH et BRIDGES [10], ont montré que le même phénomène s'observe également avec des antigènes protidiques. Cet état de "paralysie" immunitaire peut être obtenu très régulièrement avec des doses de l'ordre de milligrammes chez le Mammifère nouveau né. Le degré et la durée de cette tolérance dépendront de la nature de l'antigène, de la

dose initiale et, éventuellement, de la répétition ultérieure des injections de l'antigène.

Mais un état de non réactivité immunitaire peur être également créé chez des animaux adultes. La différence réside dans la dose d'antigène à administrer.

Ce qu'il y a lieu de souligner dès maintenant c'est que, pour induire un état de tolérance (ou de paralysie) immunitaire, il faut administrer

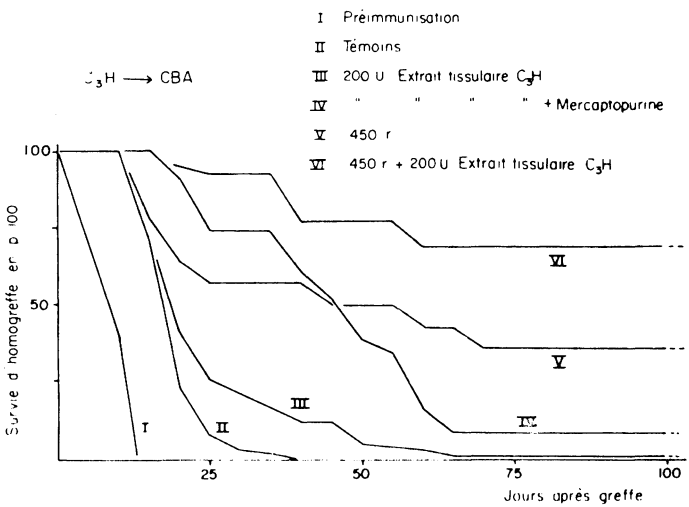


Fig. 1.—Survie d'homogreffe dans la combinaison génétique $C_3H \rightarrow CBA$ dans diverses conditions expérimentales: I. Animaux ayant subi une préimmunisation par une greffe préalable de peau de C_3H. II. Animaux CBA greffés pour 1ère fois avec la peau de C_3H (témoins). III. Idem après injection préalable d'extrait tissulaire splénique de C_3H. IV. Idem que III chez les animaux ayant reçu, en plus, de la mercaptopurine. V. Survie de greffe chez les aimaux CBA ayant reçu une irradiation avec 450 r. VI. Idem que V plus injection préalable de 200 u. d'extrait tissulaire C_3H (voir référence 4).

une dose déterminée d'antigène dont la grandeur varie selon la nature chimique de celui-ci et l'âge de l'animal. Cette notion de *quantité* d'antigène est une donnée essentielle. Nous verrons qu'elle intervient aussi bien dans le cas de la tolérance immunitaire induite avec des cellules vivantes, que lors de l'administration des antigènes solubles.

Il existe une autre forme d'inhibition immunologique qui, probablement, s'apparente, par son mécanisme, à la précédente, c'est le phénomène décrit par SULZBERGER [11] et CHASE [12]. Il est relativement facile de provoquer, chez le cobaye, une allergie dermique de contact par l'application percutanée ou intradermique de diverses substances chimiques: arsphénamines, chlorure de picryle, dinitrochlorobenzène, etc. Cette sensibilisation cutanée peut être inhibée complètement lorsqu'on administre préalablement à l'animal, soit par voie gastrique, soit par voie parentérale, la substance sensibilisante. L'état réfractaire ainsi créé

peut persister très longtemps et est spécifique pour la substance chimique donnée.

* * *

Ce qui se dégage des faits rapportés, c'est qu'il est possible de créer régulièrement, chez le Mammifère, un état d'inhibition immunologique se traduisant par une non-réactivité de l'animal à des substances régulièrement antigéniques, que celles-ci soient des cellules vivantes allogéniques, des antigènes non figurés ou encore des substances chimiques simples.

L'induction de cet état immunitaire n'est pas restreint à l'âge embryonnaire ou périnatal. Elle peut être obtenue à tout âge et elle est uniquement fonction de la dose d'antigène administrée.

Caractères de la tolérance immunitaire

Inhibition centrale.

L'état de tolérance immunitaire n'est pas lié à une modification acquise par les cellules de métaboliser l'antigène. Il reflète une inhibition centrale, c'est à dire une détérioration réelle du dispositif immunologique impliqué dans la production d'anticorps.

A cet égard je voudrais citer quelques résultats obtenus tout récemment dans mon laboratoire [15]. L'objet de ces expériences (*) était de prouver que l'induction de la paralysie immunitaire a pour conséquence non seulement l'inhibition de la production d'anticorps "in vivo", mais aussi de montrer que les cellules lymphoïdes, prélevées chez ces animaux, ont perdu la faculté de synthétiser des anticorps en culture "in vitro".

La paralysie immunitaire a été induite par l'administration d'une dose de gammaglobulines humaines (GGH) et la sensibilisation par l'injection soit des gammaglobulines bovines (GGB) picrylées, soit de sérumalbumine de cobaye (SAC) picrylée, incorporées dans les adjuvants de Freund. Les anticorps ont été dosés par la technique d'hémagglutination passive à la benzidine.

Le tableau I montre que, au 9e jour après la sensibilisation, tous les animaux témoins synthétisent des anticorps circulants. Le titre moyen est de 820 chez les animaux sensibilisés avec la SAC pic. et de 1500 chez les animaux sensibilisés avec la GGB pic.

Le tableau II réunit les données concernant la production d'anticorps "in vitro".

Ces résultats montrent que les cellules lymphatiques, prélevées chez les animaux en état d'immunoparalysie par surcharge protidique, sont incapables de synthétiser des anticorps "in vitro".

(*) Dans ces expériences, l'immunoparalysie a été induite par un antigène non apparenté avec l'antigène sensibilisant (voir plus loin: Spécificité de la tolérance immunitaire).

TABLEAU I

Inhibition de la production d'anticorps circulants "in vivo" par un traitement immunoparalysant à l'aide de GGH.
Les animaux ont été sensibilisés soit avec les GGB soit avec la SAC pic.

Antigène immunisant	Titre d'hémagglutination	
	Témoins	Traités par les GGH
GGB pic.	512	—
	1024	—
	1280	—
	1280	—
	2560	—
	2560	—
	1280	—
Moyenne	1500	—
SAC pic.	512	—
	1280	—
	1024	—
	256	—
	1024	—
Moyenne	820	—

TABLEAU II

Taux d'anticoprs synthétisés "in vitro" par les explants de ganglions lymphatiques prélevés le 9° jour après la sensibilisation chez des cobayes témoins et des cobayes ayant subi un traitement immuno-paralysant.
Les animaux ont été sensibilisés avec les GGB pic.

Traitement	Jours de culture			
	3	6	9	12
0 (témoins)	60 *	120	40	8
	160	240	80	8
	256	256	16	16
	160	360	60	32
	—	360	80	16
	172	384	32	16
	160	80	60	8
Moyenne	161	257	51	15
GGH	20	20	8	—
	10	10	8	—
	10	10	4	—
	< 20	< 20	< 20	—
	< 20	< 20	< 20	—
Moyenne	12	12	7	—

(*) Les chiffres indiquent l'inverse de la dilution maximum [illegible]

Ces expériences apportent la preuve que l'immunoparalysie, induite par l'injection d'une dose élevée d'antigène soluble, est bien due à une altération du dispositif biochimique cellulaire impliqué dans la synthèse des anticorps, sans qu'on puisse dire si cette altération concerne le système de reconnaissance ou le dispositif de synthèse.

Aspect quantitatif

Au cours de premières recherches sur la tolérance immunitaire, l'aspect quantitatif du problème n'est pas apparu avec évidence et

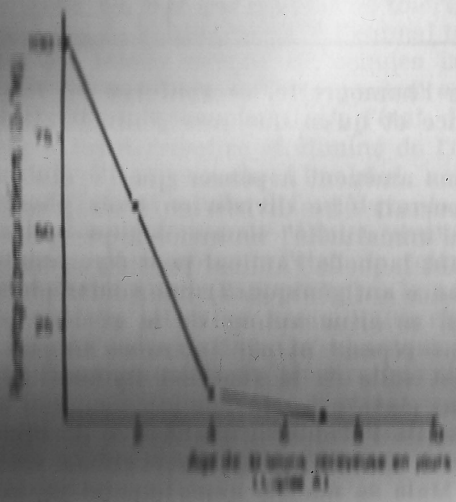


Fig. 1. Tolérance immunitaire évaluée par la durée de survie de l'homogreffe de peau en fonction de l'âge. La tolérance a été induite par l'administration, à des souris de la lignée A, de 8 millions/g. de cellules spléniques de l'hybride (CBA × A) (d'après Brent) (4). TMS : Temps moyen de survie de la greffe cutanée.

l'accent a été mis sur la *période de la vie* au cours de laquelle cet état immunologique peut être induit. On a observé que la tolérance immunitaire peut être induite aisément par l'injection de cellules vivantes homologues chez le foetus ou chez le nouveau-né immédiatement après la naissance.

En étudiant par la même méthode, jour par jour, la possibilité d'induire la tolérance immunitaire au cours de la période postnatale, Billingham et Brent [14] ont observé une modification dramatique qui se produit, à cet égard, chez la souris entre le 2° et le 4° jour qui suivent la naissance (Figure 2).

Le nombre d'animaux tolérants pour une dose de cellules, qui est de 100 p 100 chez l'animal nouveau-né, n'est plus que de 56 p. 100 au 2° jour et est voisin de zéro vers le 4°-5° jour.

Ces résultats montrent que le dispositif immunologique, qui condi-

TABLEAU III

Hypothèse sur l'évolution qualitative de l'immunité chez le Mammifère

Phase immunologique	Modalité de la réponse
Période adaptative ou immature (in utero)	Tolérance
Période neutre	Nulle
Période de maturation (adulte)	Sensibilisation

tionne le rejet de l'homogreffe, se renforce au cours des jours qui suivent la naissance et qu'en quelques jours il atteint son efficacité maximale.

Ces observations amènent à penser que l'évolution immunologique du Mammifère pourrait être divisée en trois phases: 1) une phase d'"adpatation ou d'immaturité" immunologique qui s'étend jusqu'à la vie foetale et durant laquelle l'animal peut être rendu tolérant à n'importe quelle substance antigénique. 2) une courte phase de "neutralité" immunologique qui se situe autour de la période périnatale, durant laquelle l'animal ne répond ni par tolérance ni par immunité; 3) la troisième phase est celle de la réaction immune de l'animal devenu immunologiquement mature.

Cette hypothèse de l'évolution qualitative de l'immunité, au cours de l'ontogénèse, est basée sur les observations réalisées à l'aide de cellules vivantes. Mais ce modèle expérimental ne se prête pas à une étude quantitative pour deux raisons: la composition antigénique des cellules n'est pas connue avec précision, ce qui ne permet pas de connaître le nombre d'antigènes injectés ni leur quantité; de plus, les cellules vivantes injectées continuent à se multiplier dans l'organisme de l'hôte immunologiquement immature, ce qui rend encore plus malaisé l'évaluation quantitative des antigènes présents.

L'étude réellement quantitative, réalisée à l'aide des antigènes solubles tels que les antigènes protidiques et polysaccharidiques, a montré que la tolérance immunitaire n'est pas un phénomène qualitatif et qu' elle répond à des lois quantitatives. Il existe, pour chaque antigène, une dose critique, qui varie selon la nature de l'antigène et selon l'espèce animale. Au-dessous de cette dose critique l'organisme répond par une réaction de sensibilisation, alors qu'au desssus de cette dose, on obtient régulièrement la tolérance immunitaire. La dose critique est indépendante de l'âge et elle semble être en rapport, d'une part, avec la vitesse du métabolisme de l'antigène et, d'autre part, avec la masse des cellules immunologiquement compétentes.

On a pu ainsi calculer que, chez la souris, la dose de polyosides de Pneumocoque, nécessaire pour induire la tolérance immunitaire est de

5 mg/g.[15] et celle des gammaglobulines de boeuf de 0,5 mg/g.[16], quel que soit l'âge de l'animal. Le même phénomème s'observe avec des cellules vivantes. HOWARD, MICHIE et WOODRUF[17] ont, en outre, montré que si l'on injecte, à des souris nouveau-nées, environ 200.000 cellules spléniques, on obtient un état de sensibilisation mais qu'avec une dose de cellules de l'ordre de 5 millions, on obtient un effet de tolérance.

D'autre part, on s'est aperçu que l'état de tolérance immunitaire n'est pas un phénomène définitif, mais un processus dynamique. Sa durée est étroitement liée à la persistance de l'antigène dans l'organisme. Lorsqu'on a recours à des cellules vivantes, la tolérance ainsi induite peut persister longtemps, parfois toute la vie, puisque les cellules auto-reproductrices continuent à maintenir dans l'organisme indéfiniment un taux adéquat d'antigène. On sait que cet état de tolérance peut toutefois être obtenu à volonté par l'administration, à l'animal tolérant, de cellules isologues normales ou, mieux encore, de cellules isologues prélevées chez un animal préalablement sensibilisé au même antigène.

Lorsqu'on utilise des antigènes solubles, l'état de tolérance prend fin quand l'antigène a été métabolisé et éliminé de l'organisme.

Le phénomène est identique chez l'animal nouveau-né et chez l'animal adulte. Chez l'animal adulte il faut davantage d'antigène, que celui-ci soit administré sous forme de cellules vivantes ou sous forme de substances solubles, pour obtenir l'état de tolérance. HOWARD et collaborateurs[11] ont établi que le rapport entre le nombre des cellules, nécessaires pour obtenir la tolérance immunitaire chez l'animal adulte et le nouveau-né, est de l'ordre de 200. Qu'est-ce qui détermine la grandeur de ce facteur? Il est plausible d'admettre que c'est le nombre respectif des cellules immunologiquement compétentes de l'hôte. Il est intéressant de souligner que le nombre des cellules lymphocytaires, dans la rate d'une souris adulte, est environ 200 fois plus grand que celui de la souris nouveau-née.

Ainsi la réponse de l'hôte sera du type "sensibilisation" ou "tolérance" selon que le rapport entre la dose d'antigène et le nombre de cellules compétentes sera au-dessous ou au-dessus de la valeur critique. Chez l'animal adulte, il faut, de toute évidence, une dose d'antigène plus grande pour disposer des cellules compétentes de l'hôte. C'est ce que j'ai essayé de schématiser dans la figure 3.

Comment expliquer le phénomène? Deux hypothèses peuvent être envisagées. La première est que l'excès d'antigène tue les cellules immunologiquement compétentes *(mort allergique)*. La seconde est que les antigènes administrés produisent un double effet: ils sensibilisent les cellules immunologiquement mûres et ils induisent un état de tolérance "active" des cellules jeunes ou néoformées. Le maintien d'un taux convenable d'antigène amène, petit à petit, un déplacement dynamique de la population cellulaire dans le système, la population de cellules sensibilisées étant progressivement remplacée par celle de cellules tolérantes.

Ainsi la tolérance immunitaire n'est pas une propriété absolue, liée à la maturité immunologique de l'animal. Elle n'est pas davantage un phénomène définitif. A tout âge, le Mammifère peut réagir à l'introduc-

tion d'un antigène, quelle qu'en sois la forme par une réaction "sensibilisation" ou par celle de "tolérance". La nature de la réaction dépend de la dose d'antigène ou plutôt du rapport entre la dose d'antigène et le nombre de cellules immunologiquement compétentes.

Immunité et *tolérance* sont donc deux réponses *qualitativement* différentes mais liées par un facteur quantitatif.

Spécificité.

La spécificité de la tolérance ou de la paralysie immunitaire est admise par presque tous les auteurs, car elle est étayée par una expérimentation aussi ample que variée.

Les expériences classiques de MEDAWAR et collaborateurs [1] ont

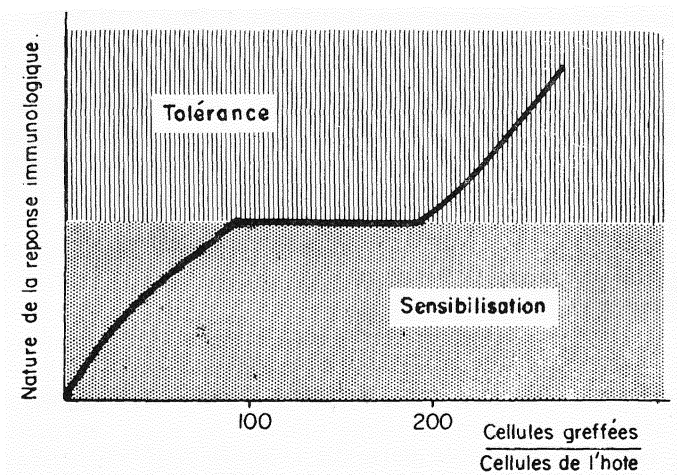


Fig. 3.—Représentation schématique de la nature de la réponse immunologique en fonction de la quantité de cellules immunologiquement compétentes injectées.

établi que, seule, la peau provenant de la souche donneuse des cellules spléniques injectées au receveur à la naissance, est acceptée par celui-ci.

FELTON [18] a observé que des souris rendues tolérantes à un polysaccharide d'une souche de Pneumocoque peuvent être immunisées avec un autre polysaccharide d'un autre type. SMITH et BRIDGES [10] ont souligné la spécificité de la paralysie immunitaire induite avec des protéines sériques. Cependant les expériences réalisées avec LIACOPOULOS, indiquent que cette notion doit être révisée [19, 20].

Il ressort de ces observations que l'administration, à des cobayes adultes, de doses élevées et répétées d'un antigène soluble, —il s'agit, en l'occurrence, de protéines sériques— rend l'animal réfractaire à des doses *sensibilisantes* d'un autre antigène, tel que l'ovalbumine. Cette paralysie immunitaire affecte non seulement la synthèse d'anticorps circulants mais également l'hypersensibilité du type retardé.

Dans cette dernière partie de l'exposé, je désire rapporter, avec quelques détails, les résultats de ces recherches et en analyser le mécanisme.

Hypersensibilité du type immédiat

A des cobayes adultes on a administré de la SAB à une dose de 500 mg/kg. et par jour, soit par voie intraveineuse soit par voie intrapéritonéale. Ce traitement détermine, après quelques jours de traitement, un état de paralysie immunitaire vérifiée par l'absence de produc-

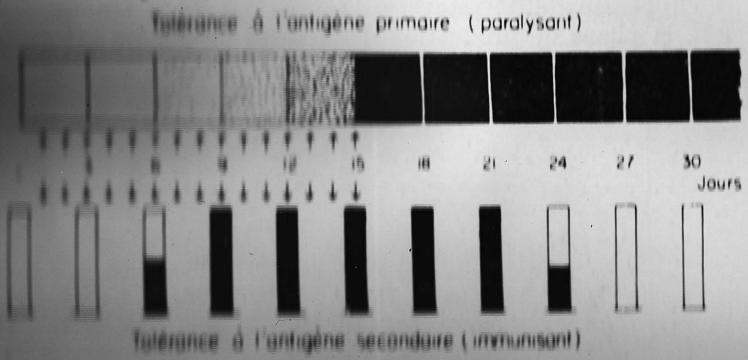


Fig. 4. Représentation schématique de l'installation de la paralysie immunitaire non spécifique chez des cobayes adultes sous l'effet d'injections répétées de doses élevées de SAB par voie intraveineuse. En blanc: réponse immunologique normale. En gris: paralysie partielle à l'égard de l'antigène secondaire. En noir: Absence de réponse à une dose immunogène de l'antigène secondaire.

tion d'anticorps anti-SAB, même deux mois après l'arrêt du traitement. Durant cette période de traitement, on administre un autre antigène, en l'occurrence soit de l'ovalbumine, soit la gammaglobuline de lapin, précipitées à l'alun.

Chez les animaux normaux l'injection de ces antigènes produit un état de sensibilisation, caractérisé par l'apparition de manifestations anaphylactiques et de taux élevés d'anticorps sériques.

Chez les animaux traités par la SAB, les résultats observés varient selon certains paramètres expérimentaux. Si l'antigène "sensibilisant" (ovalbumine ou gammaglobuline de lapin) est administré simultanément avec la première injection de l'antigène "paralysant" (sérumalbumine de boeuf à fortes doses), les animaux traités se sensibilisent au même titre que les témoins. Mais si l'antigène "sensibilisant" est administré quelques jours après le début du traitement paralysant et par la même voie, on constate, au fur et à mesure que l'intervalle de temps entre ces deux traitements augmente, que le pourcentage d'animaux qui se sensibilisent décroît progressivement. Lorsque cet intervalle de temps est de 10 jours ou davantage, aucun des animaux traités ne se sensibilise à l'antigène immunogène (Figure 4).

Ainsi il est possible d'induire, en soumettant des animaux adultes à des injections de doses élevées et répétées d'une protéine hétérologue, un ètat immunologique qui se reflète non seulement par l'abolition de la réponse immune à l'égard de cet antigène, mais aussi à l'égard d'autres substances antigéniques, chimiquement non apparentées, administrées à des doses immunogènes.

Hypersensibilité du type retardé

On sait les relations qui existent entre l'hypersensibilité conventionnelle et l'hypersensibilité retardée. D'après SALVIN [21], UHR et PAPPENHEIMER [22], la phase de production d'anticorps circulants est régulière-

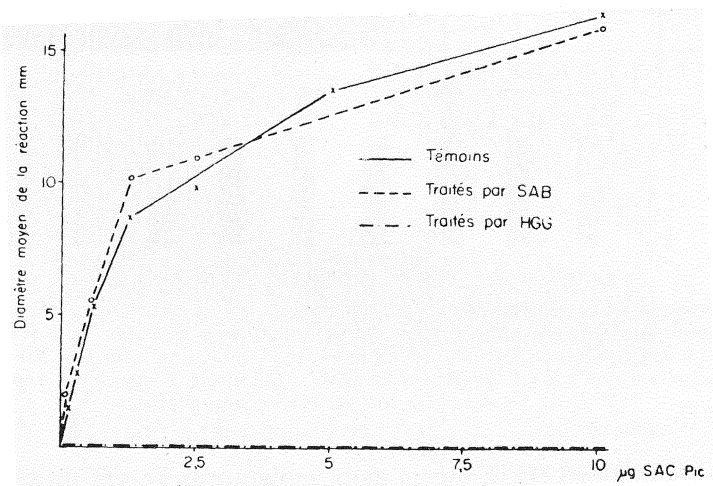


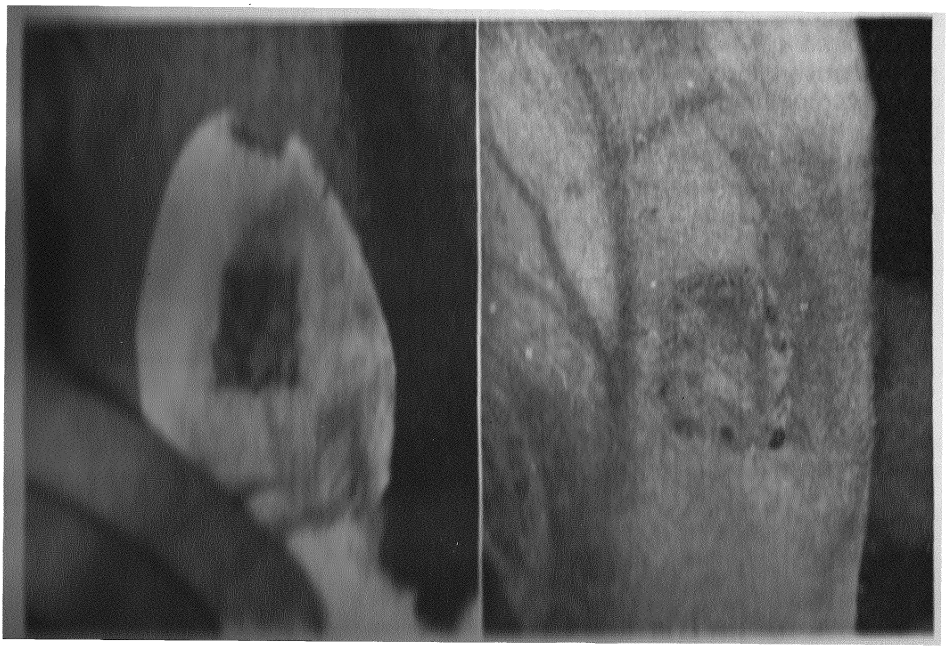
Fig. 5.—Degré d'inhibition de l'hypersensibilité retardée (mesuré par le diamètre de la réaction dermique) chez des animaux sensibilisés avec la SAB pic. de cobaye incorporée dans les adjuvants de Freund par les injections répetées de doses élevées, soit de SAB de boeuf, soit de gammaglobulines humaines (Inefficacité à peu près complète de la SAB bovine, alors que les gammaglobulines humaines inhibent complètement la réaction retardée à la SAB pic. de cobaye).

ment précédée d'une phase plus ou moins prolongée d'hypersensibilité retardée à cet antigène. Il nous a paru intéressant d'étudier l'évolution de l'hypersensibilité retardée à un antigène différent, chez des animaux soumis à un traitement immunoparalysant.

Sans vouloir entrer, ici, dans des détails qui ont été rapportés ailleurs [19], [20], je me contenterai de préciser les résultats essentiels. Une inhibition de l'hypersensibilité retardée a pu être observée chez des cobayes recevant des doses élevées de protéines sériques hétérologues, à l'égard d'un antigène immunologiquement non apparenté, administré à des doses immunogènes. Cet effet était surtout évident avec les gammaglobulines hétérologues, et lorsque le traitement général

[illegible] associé avec un traitement local, c'est à dire la [illegible] que l'antigène immunisant. L'action de la sérumal[illegible] (Figure 5).

[illegible] cette inhibition de l'hypersensibilité retardée [illegible] la prolongation de l'homogreffe cutanée que



[illegible]

[illegible] Lapin recevant des doses élevées de gamma[illegible] par voie sous-cutanée, dans la zone où la greffe [illegible] (Figure 6).

[illegible] non spécifique du pouvoir sensibilisant, induite par [illegible] doses élevées de protéines hétérologues effective que [illegible] du traitement immuno-paralysant. Lorsque l'injection [illegible] immunisant est faite 15 jours après l'arrêt du traitement [illegible] paralysant, la sensibilisation a lieu et elle ne diffère pas de [illegible] des témoins.

Une confirmation de ces faits est apportée par les observations faites sur l'hypersensibilité retardée. Celle-ci, avons-nous vu plus haut, est totalement inhibée au 7e jour après la sensibilisation, chez les animaux soumis au traitement immuno-paralysant. Mais sur ce phénomène également l'effet inhibiteur est passager, et on voit, après cessation du traitement immuno-paralysant, la réactivité de l'animal à l'antigène

réapparaître lentement pour atteindre la même intensité que chez les témoins, environ deux mois après l'arrêt du traitement (Figure 7).

Conclusions.

La tolérance immunitaire, induite chez le Mammifère à l'aide de cellules compétentes, et la paralysie immunitaire, créée par l'administration d'antigènes solubles, ne sont que deux variantes du même phénomène biologique. Celui-ci traduit la perte de la capacité de l'organisme

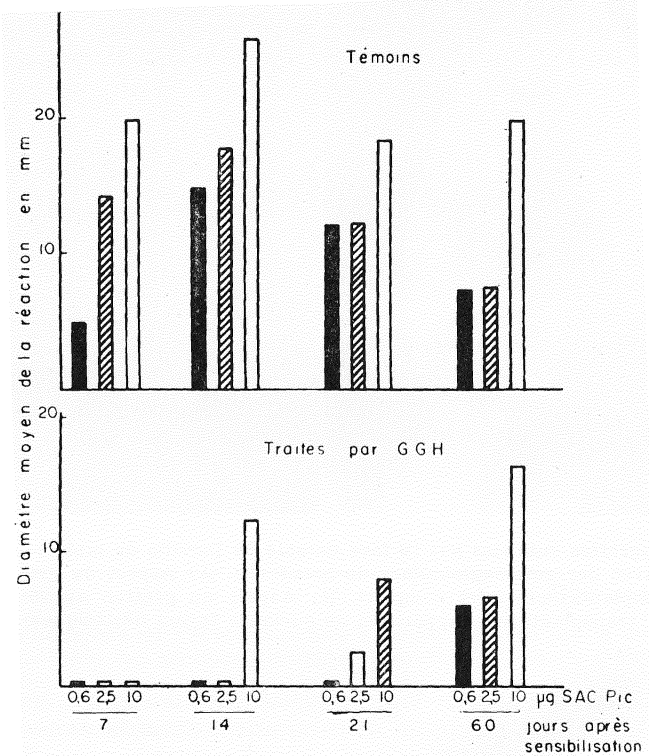


Fig. 7.—Intensité de la réaction d'hypersensibilité retardée à des doses différentes de SAB pic. de cobaye chez les animaux sensibilisés à la SAB pic. de cobaye. En haut: animaux témoins. En bas: animaux traités avec les gammaglobulines humaines.

de réagir, par une réponse immune habituelle, aux substances douées de propriétés antigéniques.

L'inhibition immunologique ainsi créée est d'origine centrale ainsi que le prouve l'incapacité des cellules des animaux tolérants de synthétiser des anticorps correspondants "in vitro".

La tolérance immunitaire n'est pas l'apanage des animaux nouveau-nés et elle peut être régulièrement induite chez l'animal adulte.

La différence réside dans la quantité d'antigène requise, en relation avec la population des cellules immunologiquement compétentes.

La tolérance immunitaire n'est pas une propriété définitive. Cet état dynamique peut être abrogé soit par l'introduction de cellules immunologiquement compétentes isogéniques vierges ou pré-immunisées, ou encore lors de l'élimination de l'antigène.

La durée de la tolérance immunitaire est fonction de la présence de l'antigène dans l'organisme. La tolérance immunitaire est généralement considérée comme étant d'une spécificité aussi rigoureuse que l'immunité. Cependant, des travaux récents, réalisés dans mon laboratoire et qui sont analysés ici, indiquent qu'une surcharge prolongée en antigène hétérologue est susceptible de créer une inhibition immunologique s'étendant durant une période déterminée à des antigènes immunologiquement non apparentés avec l'antigène immuno-paralysant.

Les modalités du phénomène de paralysie immunitaire non spécifique sont analysées.

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ALERGIA EXPERIMENTAL

EXPERIMENTAL ALLERGY

ALLERGIE EXPÉRIMENTALE

EXPERIMENTELLE ALLERGIE

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HYPERSENSITIVITY INDUCED IN MAN WITH EMULSIFIED ANTIGENS (*)

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In 1950, while studying the rate of absorption of a labeled pollen extract emulsified in mineral oil-Arlacel adjuvant, we noted that 5 of 6 nonatopic persons developed a delayed skin hypersensitivity to that antigen [1]. It appeared to us that the use of adjuvant-emulsions of such poor antigens might constitute a good tool in the study of the mechanism and development of hypersensitivity in man.

For obvious reasons, including the fact that one of our medical student subjects developed a sterile abscess, we transferred our investigation to a prison population. The majority of nonatopic persons (27 out of 47), injected with emulsified pollen, developed this delayed skin hypersensitivity, which was definitely attributable to the pollen extract and not to any other ingredients added. This sensitivity was shown by the production of a delayed inflammatory reaction at the site of an intradermal injection beginning in 6 to 24 hours and lasting one to several days or weeks. The degree of reactivity varied from mild to intense (even with necrosis in some instances) and extended to as dilute extracts as 1:1000000. Those with the higher degree of induced delayed sensitivity evidenced a gradually developing swelling and heat at the site of the injection of the emulsified antigen, which, in the most severe instances resulted in a sterile abscess. It is significant to note that 9 sterile abscesses occurred when the emulsified pollen antigen was injected in 53 nonatopic persons, whereas out of a series of several thousand injections of emulsified antigens prepared and administered in the same manner and by the same personnel to patients who were spon-

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Associates who deserve credit for carrying out a large part of this work are (in alphabetical order and in present locations): Robert J. Becker (Joliet, Illinois), Alan R. Feinberg (Winnetka), Jordan N. Fink (Northwestern), Arthur Kaminker (Toronto, Canada), Roy Patterson (Northwestern), Jacob J. Pruzansky (Northwestern), Haskell I. Rabinowitz (McAllen, Texas), Raymond G. Slavin (Winnetka), Dale B. Sparks (Riverside, California), and James I. Tennenbaum (Lackland Air Force Base, Texas).

taneously allergic to the antigen, not a single case of abscess resulted.

The state of delayed hypersensitivity began usually 2 to 4 weeks after the emulsion injection. It tended to persist and in some cases tested was still there after 1 1/2 years. Peripheral leucocytes of this induced sensitivity, when injected i. m. and i. d., transferred this delayed hypersensitivity state to distal sites of recipients [2]. Quantitative titration (3 fold serial dilutions of the antigen) of the recipients in all instances showed a lesser degree of sensitivity than in the donors. Successive transfers (recipient to a second recipient) were not successful. The serum of delayed sensitivity donors was incapable of transfer. Punch biopsies of the sites of delayed skin reaction of donors and recipients taken at successive intervals after the injection showed a delayed onset of perivascular infiltration of mononuclear cells compatible with the cytologic picture of delayed hypersensitivity. Of the group of 47 nonatopic persons, 18 developed an immediate hypersensitivity reactión, and 8 both immediate and delayed.

Atopic individuals who were injected with emulsions of antigens to which they were spontaneously sensitive never showed a delayed reaction to that antigen. A moderate increase in immediate reactivity would be difficult to show, although CONNELL and SHERMAN [3] claim to have demonstrated an increase in reagin titer after injection of emulsified antigen. However, if atopic persons are injected with an emulsion of an antigen to which they have no spontaneous sensitivity about 95 % (32 out of 34 in one series) develop an immediate sensitivity to that antigen. Neither delayed nor immediate hypersensitivity results from one or a number of injections of aqueous extracts of these antigens. The immediate hypersensitivity was transferrable by the serum, but not by the cells, to the skin of other subjects. The sensitizing capacity was destroyed by heat and the sensitized area was reactive for 2 weeks or longer [4]. Conjunctival instillation of pollen produced an immediate reaction in some of the immediate skin reactors, but nasal application did not provoke any visible effects. Immediate reactivity took a little longer to develop than the delayed, but had about the same tendency to persist. Of the 34 atopic subjects 14 also developed delayed reactivity and 14 a combination of both. Some developed delayed sensitivity as the first reaction, some immediate and some both at the same time.

The failure to induce a delayed hypersensitivity skin reaction in the atopic person by an injection of an emulsified antigen to which they were already spontaneously sensitive raised a number of possibilities. That the atopic person is constitutionally capable of developing delayed hypersensitivity was demonstrated by producing a delayed reactivity in 8 out of 19 such subjects after an injection of an emulsified pollen to which they were not previously sensitive [5]. The local reaction produced by the incorporation into the emulsion of histamine or the pollen antigen to which the atopic subjects were sensitive, did not prevent the development of delayed reactivity to the new antigen.

The question arises: Is the antigen consumed in the immediate an-

[illegible] antibody reaction, and thus is unavailable for producing the delayed reaction? In other words, might a transfer factor in the peripheral lymphocytes be present without capability of being shown directly in the actively sensitised subject? Of the 10 subjects who had a single injection of an emulsion of the pollen to which they were spontaneously sensitive, 4 were capable of transferring a delayed sensitivity reaction with their peripheral leucocytes though no delayed reactivity could be elicited in their own skin [5]. None of the 6 untreated hay fever subjects or 14 who had been treated with aqueous extracts possessed this cell transfer capability. Three delayed ragweed reactors were passively transferred with reaginic serum. An intradermal injection of the passively transferred site produced an immediate reaction, but no delayed, while a site which had not been injected with reaginic serum gave a typical delayed reaction. That this reaction is a specific one can be surmised from the fact that in subjects with delayed trichophyton and immediate ragweed sensitivity only moderately reduced trichophyton reaction resulted when both antigens were injected together intradermally.

It is recognised that some obvious observations challenge the above findings. For example, there is *spontaneous* delayed and immediate reactivity occurring to the same antigen, such as with trichophytin. Furthermore, as we have mentioned previously in a number of instances of *induced* sensitivity both immediate and delayed reactivity resulted. Possibly these discrepancies could be due to different antigens in the crude extracts. At any rate, this aspect requires further investigation. Perhaps related to this question is the preliminary finding that a purified ragweed pollen fraction has lesser capability of giving a delayed reaction than did the crude extract.

The possibility that atopic-like symptoms (such as nasal stuffiness and rhinorrhea) might occur in some instances on a delayed hypersensitivity basis is suggested by several observations. These are the frequent absence of immediate skin reactions in many suspected allergic manifestations, the not infrequent absence of eosinophile increase, the common therapeutic failure of antihistamines, and the report by some, such as Warren Vaughan, that delayed skin reactions occur in allergy. In experiments conducted by SIMON and RACKEMANN [7] about 30 years ago, nasal symptoms, delayed in onset, were produced with a concomitant delayed intradermal reaction after several weekly packings of the nose with guinea pig serum.

We repeated [8] SIMON and RACKEMANN'S experiments, as well as making nasal tests in the subjects who had developed a delayed skin reaction following an injection of emulsified pollen. Eight nonatopic subjects received weekly injections of 1:10 guinea pig serum, and 5 of these developed delayed skin reactions. Two of these developed nasal stuffiness and rhinorrhea 6 to 12 hours after a 10 minute nasal pack of 1:10 guinea pig serum. Of 12 atopic subjects, 7 developed a delayed skin test and 4 of these showed a nasal reactivity. When the sensitizing dose was used nasally in 7 nonatopic subjects neither a nasal reaction nor a skin reaction developed. However, of 14 atopic subjects who had

repeated nasal packs 8 developed nasal symptoms delayed in onset and 4 of these showed a positive delayed reaction on intradermal challenge. In the nasal reactors the nasal smears at 24 hours showed a preponderance of mononuclear cells and no eosinophiles.

Twelve nonatopic subjects who had developed a delayed skin reactivity to pollen as a result of an injection of emulsified pollen extract were challenged with a nasal pack of the aqueous pollen extract. The opposite side of the nose was packed either with the extracting fluid or with a pollen extract to which the subject had not been sensitized. The procedure was conducted on a double-blind basis. Nine of the 12 had distinct symptoms on the side of specific challenge. Nasal stuffiness or rhinorrhea began 6 hours or later and disappeared in 48 hours. Nasal smears and punch biopsies of the mucosa showed virtually no change before 6 hours. At about 6 hours symptoms began, the turbinates were slightly edematous and some polymorphonuclear neutrophiles were present in the secretion and in the biopsy. At 24 hours the turbinates were edematous, the nasal secretion showed increase in mononuclear cells and the biopsy showed many mononuclear cells, mainly perivascularly. At 48 hours all findings had reverted to practically a normal state.

The possible clinical implications of some of these findings are self-evident. We realize, however, that there are some disturbing and puzzling features. For example, the rapid reversal of the delayed nasal reaction differs considerably from what one might expect in a delayed reaction. The absence of itching and sneezing in the nasal symptomatology is striking. And there are other features on which enlightenment is needed before conclusions can be drawn.

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EXPERIMENTAL HYPERSENSITIVITY TO SIMPLE CHEMICAL HAPTENS

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After further consideration of 'carrier specificity' and its possible importance in clinical sensitization to simple chemicals, the type of sensitivity exhibited by guinea pigs showing delayed reactivity to hapten-protein conjugates will be discussed, with special reference to the results of desensitization. Some theoretical problems as to the nature of the desensitization process in delayed sensitivity will be raised.

IMMUNOCHEMICAL MECHANISMS INVOLVED IN PENICILLIN. HYPERSENSITIVITY IN EXPERIMENTAL ANIMALS AND IN HUMAN BEINGS

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In addition to its importance in clinical medicine, the human penicillin allergy system provides a comparatively well defined haptenic system for the study of fundamental immune mechanisms involved in human allergic diseases. Also, the understanding of the mechanisms involved in clinical allergic reactions to penicillin would in turn allow for more rational approaches to the prediction, diagnosis prevention and management of these diseases. Penicillin allergies are, of course, immune diseases, i. e., they follow antibody/antigen reactions taking place in relation to tissue. Accordingly, the following kinds of information are important in elucidating their immune mechanism: 1, the chemical mechanisms of antigenicity of penicillin; 2, the antigenic specificities of immune responses to penicillin; 3, the kinds and quantities of antibodies synthesized in response to the administration of penicillin and; 4, the relationship of the nature of the immune responses to the occurrence of clinical allergic reactions. Studies in experimental animals provide a basis for later studies in human beings, and broaden the biological significance of the results obtained. This paper will deal with the chemical mechanisms of antigenicity of penicillin, the antigenic specificities of the immune responses of guinea pigs, rabbits and human beings to administration of penicillin, our early views on the possible immune mechanisms involved in clinical allergic reactions to penicillin, and the clinical implications of these views.

Antigenicity of benzylpenicillin.—In order for low molecular weight chemicals to induce immune responses, they must first combine irreversibly with tissue macromolecules (most probably, proteins) to form the hapten-protein conjugates which induce the synthesis of specific antibodies. This view is widely accepted among immunologists, and is based on much data which show that of a large number of simple chemicals, only those which combine, through covalent linkages, with proteins of with protein-model compounds are capable of inducing immune res-

With regard to many drugs which can cause allergic reactions, but would appear from their structural formulae to be incapable of reacting irreversibly with proteins, it is reasonable to postulate that either a trace contaminant, a degradation product, or an intermediate metabolite of the drug may be the actual protein-reactive material [1]. With regard to the antigenicity of benzylpenicillin (penicillin G, PG), the available evidence indicates that PG first rearranges to form an

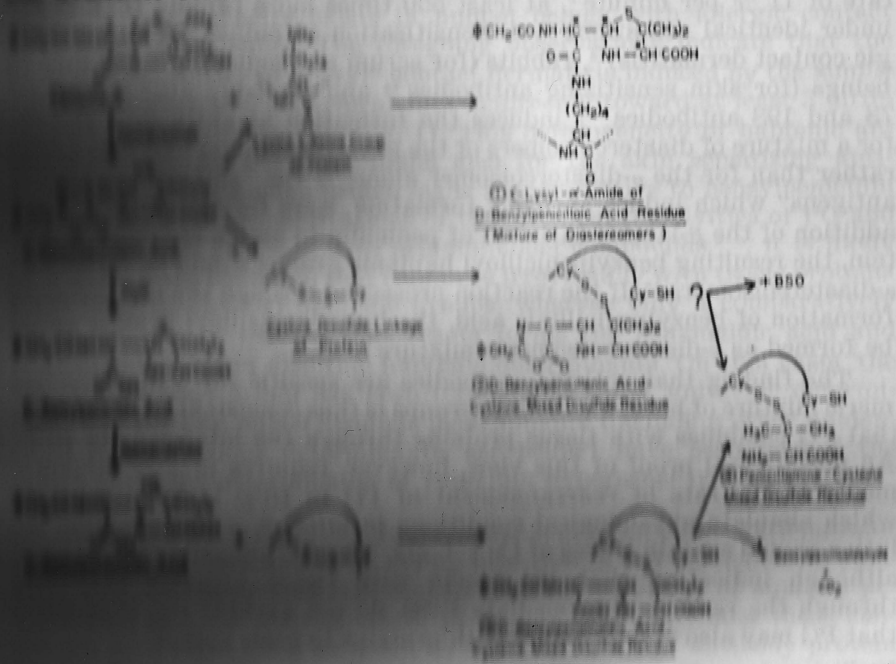

Fig. 1. The proposed pathways which may be involved in the *in vivo* formation of the penicillin antigen by reaction of PG with epidermal proteins (4).

isomer, D-benzylpenicillenic acid (BPE) (fig. 1), which is a highly reactive compound, and which is the protein-reactive compound responsible for the induction of immune responses to PG [4, 6]. This rearrangement appears not to be dependent upon *in vivo* enzymatic catalysis. It occurs *in vitro* as well as *in vivo*, and its rate is increased by low pH and by the presence of cations such as Cu^{++}, Zn^{++}, Fe^{++} [7,8]. BPE reacts irreversibly mainly with lysine—amino groups of protein to form benzylpenicilloyl—amine haptenic groups [6, 9] (fig. 1). Other haptenic groups are formed to a lesser extent, as will be taken up below. Alternatively, it may be considered that PG might react to form benzylpenicilloyl haptenic groups by a direct addition reaction of the β-lactam carbonyl to lysine amine residues of proteins. This view of the antigenicity of PG appears less likely from the following lines of evidence: 1. Although this latter reaction does proceed at pH 11.5 with a half life in the order

of minutes [10], it would proceed very much slower at pH 7.5, probably in the order of 1/10,000th as rapidly as at pH 11.5. 2. PG does rearrange to BPE under physiological conditions *in vitro* [5, 8] and undoubtedly also *in vivo*. The rate of rearrangement *in vivo* is unknown. Based on the data of Eagle [11] and on arguments given previously [4], it may be in as much as of 10 % per hour. 3. BPE is an exceedingly chemically reactive compound. In aqueous solution, pH 7.5 at 37°, it is hydrolyzed at the rate of 11 % per minute [6], at least 550 times more rapidly than is PG under identical conditions [12]. 4. Sensitization of guinea pigs (for allergic contact dermatitis) [4], rabbits (for serum antibodies) [9, 13] and human beings (for skin sensitizing antibodies [14] and probably also for serum 7S and 19S antibodies [10]) induces the formation of antibodies specific for a mixture of diastereoisomers of the benzylpenicilloyl haptenic group rather than for the α-diastereoisomer alone. If the "complete penicillin antigens" which induce antibody formation, were formed by the direct addition of the β-lactam carbonyl of penicillin to amine residues of protein, the resulting benzylpenicilloyl haptenic groups would be formed as α-diastereoisomers [15]. If the reaction proceeded through the intermediate formation of benzylpenicillinic acid, the benzylpenicilloyl groups would be formed as φ-diastereoisomeric mixture [6].

The finding that anti-PG antibodies are specific for a diastereoisomeric mixture of benzylpenicilloyl groups is thus consistent with the view that PG combines with tissue proteins through the intermediate BPE. An unequivocal proof of this view, however, requires precise measurements of the rate of rearrangement of PG to BPE under conditions which simulate physiological conditions *in vitro,* e. g. the reaction solution should contain traces of Cu^{++} etc. These arguments given above, although indicating that PG reacts with tissue proteins primarily through the reactive intermediate BPE, do not exclude the possibility that PG may also react directly with proteins to some extent.

Antigenic specificities of immune responses to PG in experimental animals.—Guinea pigs can be made allergic (contact dermatitis type) to PG by repeated percutaneous application of solutions of PG in a solvent containing the anionic detergent Tween 80 [4].

The haptenic specificities of allergic contact dermatitis reactions were inferred from the patterns of allergic cross-reactivity among PG and its highly purified degradation products, as well as from the chemical reactivities of PG and its degradation products. The observation that groups of guinea pigs sensitized with PG or with BPE both give identical allergic reactions to patch tests with PG and with BPE (i. e., the two groups of guinea pigs are "allergically equivalent" see ref. 4 for more detailed descriptions) indicates that the allergic contact reactions are specific for the diastereoisomeric benzylpenicilloyl groups. In addition, specific allergic cross reactivity among PG, sodium benzylpenicilloate and D-penicillamine is observed [4]. These data and the finding that benzylpenicilloate reacts wtih cystine (through the intermediate formation of benzylpenamaldic acid-cysteine mixed disulfide) to form

D-penicillamine cysteine mixed disulfide [10] (fig. 1), indicates that the benzylpenamaldic acid-cysteine mixed disulfide and the D-penicillamine cysteine mixed disulfide groups are also haptenic determinants. In addition, the benzylpenicillenic acid-disulfide group (fig. 1) is a possible fourth haptenic determinant. In the few studies done on human beings, two of 5 patients with allergic contact dermatitis to PG reacted also in patch tests with D-penicillamine [18], which suggests that a similar haptenic heterogeneity exists in human beings with allergic contact dermatitis to penicillin. The foregoing observations indicate that the antigenic specificity of allergic contact dermatitis induced by the simple chemical compound, benzylpenicillin is directed toward at least 3 different haptenic determinants (fig. 1). This heterogeneity of haptenic antigenic specificity has been found also for the skin sensitizing antibodies induced in human beings made hypersensitive to benzylpenicillin (see below). From considerations of the chemical reactivities of PG and its degradation products with protein-model compounds [4, 6], it is likely that most of the penicillin that combines irreversibly with tissue proteins form benzylpenicilloyl haptenic groups, whereas only a comparatively small fraction of the PG that reacts forms the penamaldic acid and penicillamine mixed disulfide haptenic determinants. On this basis, the benzylpenicilloyl group has been termed the *major* antigenic determinant, and the two mixed disulfide determinants (including other unidentified trace haptenic determinants, see below) have been termed the *minor* antigenic determinants of penicillin hypersensitivity [14, 17].

Antigenic specificity of rabbit anti-benzylpenicillin antibodies. — Rabbits can be immunized to PG by injection of PG emulsified in complete Freund's adjuvants followed by booster injections of aqueous penicillin or aqueous suspensions of procaine penicillin. Under these conditions, sera containing as much as 400-500 ug/ml of antibody protein can be obtained [9]. These antibodies are specific for the benzylpenicilloyl haptenic group as shown by quantitative precipitin techniques [9], by passive cutaneous anaphylaxis (PCA) in guinea pigs [9, 18], and by passive hemagglutination [19]. Benzylpenicilloyl haptenic specificity of these immunological reactions was confirmed by quantitative hapten inhibition techniques [8, 15, 16] using benzylpenicilloyl-ε-aminocaproate [15] as the univalent hapten. The anti-benzylpenicilloyl antibodies show specificity also for the lysine side chain of proteins through which benzylpenicilloyl groups are predominantly bound to protein [9, 15, 20]. Other studies have demonstrated that anti-benzylpenicilloyl antibodies produced by immunisation of rabbits with benzylpenicilloyl-rabbit serum albumin conjugates are specific also for structural areas of the carrier protein (carrier specificity) [20]. Some evidence indicates that anti-benzylpenicilloyl antibodies produced by immunization of rabbits with PG also show carrier specificity to as yet unidentified carrier proteins [13]. Thus, available evidence indicates that the bulk of antibodies produced by immunization of rabbits with PG show immunological specificity for a rather large antigenic unit comprised of the entire benzylpenicilloyl haptenic group,

the lysine side chain, and possibly also for structural configurations of as yet undefined autologous protein carriers to which benzylpenicilloyl groups are bound. No antibodies specific for the benzylpenicillenic acid mixed disulfide or the D-penicillamine mixed disulfide haptenic groups were detected in rabbit anti-PG sera by precipitation and by PCA [9, 14], nor could indirect evidence of antibodies specific for the D-benzylpenamaldic acid-mixed disulfide haptenic group be obtained [9]. Deweck [19] stated that he has detected anti-benzylpenicillenic acid disulfide antibodies in rabbit anti-PG sera, but no supporting data was given.

Antigenic specificities of human anti-penicillin antibodies.—On an operational basis, 2 different classes of anti-PG antibodies can be detected in human beings, i. e. skin sensitizing antibodies and serum antibodies detectable by passive hemagglutination. From studies in other antigenic systems, skin sensitizing antibodies are believed to be γ1A globulins [21, 22]. Antigenic specificities of skin sensitizing antibodies can be studied by direct skin test for wheal-and-flare reactions, and by passive transfer of sera from patients with recent allergic reactions to the skin of non-sensitive human recipients.

Three kinds of antigenic specifities of skin sensitizing antibodies have been found by these studies: 1. Benzylpenicilloyl-specific antibodies were detected by direct skin tests [9, 14, 23] and by passive transfer techniques [17]. Skin test reagents which can be used for detection of benzylpenicilloyl (BPO) specific antibodies are multivalent BPO-haptens such as BPO-protein conjugates and BPO-polylysine. Succinylated BPO-polylysine conjugates of poly-L-lysine and poly-D-lysine appear, at present, to be the most useful clinical reagents for the detection of BPO-specific skin reactivity. They are effective elicitors of BPO-specific wheal-and-flare reactions [23, 14], they are virtually non-irritating, and they are incapable of inducing immune responses in experimental animals [10, 24, 25], and probably also in man. BPO-specificity of these wheal-and-flare reactions [9, 14, 17, 23] as well as specificity for a diastereoisomeric mixture of BPO groups [14] was confirmed by hapten inhibition experiments, using benzylpenicilloyl aminocaproate [15] as the univalent hapten. Other experiments indicate that the anti-BPO skin-sensitizing antibodies show specificity also for structural configurations of the autologous carrier proteins which induced the synthesis of antibody (carrier specificity) [14]. Carrier specifity toward human serum albumin was indicated in one patient [14], toward human gamma globulin in another [9], and to as yet unidentified proteins in others [14]. Since skin sensitizing antibodies may mediate some kind of allergic reaction to penicillin, the indication that anti-BPO antibodies are specific also for structural configurations of homologous proteins would suggest that some allergic reactions to penicillin are, in part, auto-immune diseases. The exact clinical significance of carrier specificity is not yet known. 2. Another group of patients show specific wheal-and-flare skin reactivity by direct skin test to crystalline potassium benzylpenicillin (KPG) and to crystalline sodium benzylpenicilloate (free from PG impurity) [10, 14]. Two of these patients (out of

[illegible] gave weak reactions also to a multivalent D-penicillamine con[illegible]. Two other patients were tested and failed to react to D-pe[illegible], penilloaldehyde and to benzyloxazolone [10]. This pattern of [illegible] indicates specificity to the D-benzylpenamaldic-cysteine mixed [illegible] and the D-penicillamine-cysteine mixed disulfide groups [14]. [illegible], this specificity must be confirmed by more direct experiments. [illegible] postulated chemical pathway leading to the formation of these [illegible] groups is shown in fig. 1, 3. The third kind of antigenic spe[illegible] was observed by passive transfer technique [11]. Human skin sites [illegible] sensitized with serum from patients with recent immediate [illegible] reactions to penicillin, gave wheal-and-flare reactions [illegible], but not to sodium benzylpenicilloate, nor to multiva[illegible] of the BPO, the D-benzylpenicillenic acid-disulfide or the [illegible] groups. The antigenic specificities of these [illegible] have not been determined. Some possibilites [illegible] in addition to these 3 antigenic specificities PAR[illegible] have reported skin sensitizing antibodies specific for the ben[illegible] mixed disulfide group, but this has not as yet been [illegible]. Accordingly, skin-sensitizing antibodies synthesized by [illegible] beings in response to the administration of PG show considerable [illegible] heterogeneity. Similar antigenic heterogeneity is seen in aller[illegible] contact dermatitis to penicillin (see above).

Hemagglutinating anti-penicillin antibodies are detected by testing [illegible] dilutions with human group O red blood cells (RBC) which had [illegible] previously reacted with PG [26], [28] or with benzylpenicillenic acid [29]. [illegible] methods of RBC sensitization result in BPO haptenic groups co[illegible] linked to the RBC membrane, probably through amide linkages [illegible] residues of membranous proteins. It is not as yet known [illegible] other of the possible penicillin haptenic groups are also coupled [illegible] by these methods of sensitization. Hemagglutinating human [illegible] antibodies are both 19S and 7S γ-globulins, with 19S antibodies [illegible] considerably more frequently [31], [30], [29]. Anti-BPO antibodies of [illegible] class may also give passive hemagglutination, but this has not [illegible] demonstrated. The haptenic specificity of hemagglutinating [illegible] antibodies is predominantly toward the BPO group [32], [29]. Other [illegible] specificities, although likely, have not as yet deen demons[illegible].

Immune mechanism of allergic reactions to penicillin.—Data perti[illegible] to this problem can be obtained from prospective and retrospective [illegible] of the immune status of patients with the occurrence of cli[illegible] allergic reactions. In these studies, it is important to consider [illegible] the different types of allergic reactions, e. g., immediate sys[illegible], late urticarial, serum-sickness-like, etc., since each kind of reac[illegible] may have distinctly different immune mechanisms. Also, it should [illegible] considered that non-immune mechanisms (i. e., vascular sensitivity [illegible] histamine, or the activity of the reticulo-endothelial system in clear[illegible] the plasma of antigen-antibody complexes) may play a role in de-

termining whether a patient in whom is present the immunological factors necessary to develop an allergic reaction, does indeed develop an allergic reaction.

It appears probable, at the present time, that immediate systemic allergic reactions to penicillin are mediated by skin-sensitizing (γ 1A) anti-PG antibodies. This statement is based on the following evidence: Siegel has reported that all of 18 patients with recent immediate systemic reactions to penicillin contained in their sera, skin-sensitizing anti-PG antibodies demonstrable by passive transfer [33]. In addition, 6 other patients with this clinical history all showed direct wheal-and-flare skin reactivity to penicillin skin-test reagents [29]. This close association of anaphylaxis and the presence of skin-sensitizing antibodies has been found in other antigenic systems in human beings [34], and in other mammalian species [35, 36]. Of interest are the findings that the antigenic specificities of skin sensitizing anti-PG antibodies from patients with immediate systemic allergic reactions were mainly to the minor antigenic determinants (i. e. skin reactions were evoked by skin test with KPG alone, or by KPG and sodium benzylpenicilloate, see above) and only comparatively rarely to the benzylpenicilloyl haptenic group [14, 17, 33]. The presence of BPO-specific skin sensitizing antibodies does not necessarily mean that a patient will develop an immediate systemic reaction on administration of penicillin. RYTEL et al. [37] reported that none of 26 patients with strongly positive wheal-and-flare reactions to penicilloyl-polylysine, and who were immediately thereafter injected with 1.2 million units of benzathine penicillin developed immediate systemic reactions, although 2 of the 26 developed late urticarial reactions. It is possible that these patients were protected from immediate systemic reactions by the presence of circulating "blocking" antibodies (7S and/or 19S) of the same haptenic specificity. No prospective studies are yet available correlating the presence of skin-sensitizing antibodies of minor antigenic determinant specificity to the occurrence of immediate systemic allergic reactions.

The immune mechanism involved in late urticarial reactions, in serum-sickness-like and in other types of late allergic reactions have not as yet been elucidated. Since only 50-80 % of such patients have detectable skin-sensitizing antibodies [14], immune mechanisms other than, or in addition to those involving the participation of skin-sensitizing antibodies may be involved.

Clinical Implications.—An important clinical application of these studies is to detect those patients who will have an allergic reaction upon the administration of penicillin. From the considerations discussed above, patients who are liable to develop immediate systemic allergic reactions should show wheal-and-flare reactions on direct skin testing with the proper antigenic reagents, providing that the patients are physiologically capable of manifesting such skin reactions. The available data on the antigenic specificites of skin-sensitizing antibodies found in patients with recent immediate systemic allergic reaction to peni-

sitin indicates that crystalline KPG may be a more effective detector of the potential immediate reactor than is penicilloyl-polylysine (see above, and [11], [11]). However, in order to detect the potential immediate systemic reactor, patients should be tested with both these reagents. A positive wheal-and-flare reaction does not necessarily mean that a patient will have an immediate system reaction on administration of penicillin, but it increases the probability of its occurrence. A negative skin reaction to both test materials appears, at present, to indicate that the patient will be able to receive penicillin without the occurrence of an immediate systemic reaction. However this view is based on theoretical considerations and on experience with a relatively small number of patients. It is still possible that other skin test materials will have to be added to those now available in order to detect very rare cases of penicillin allergy. Before negative, skin tests to the materials now at hand can be accepted as *absolutely* ruling out the possibility of an immediate systemic allergic reaction, many more patients must be evaluated. At the present time, it is not recommended that prior skin testing of patients about to be treated with penicillin be instituted as a routine clinical procedure. Before this can be done, additional information must be obtained on the sensitizing capabilities of the skin test reagents. It is to be noted, that negative skin test to the reagents will not rule out the subsequent occurrence of a late urticarial or serum sickness-like allergic reaction following the administration of penicillin. At present, there is no definite way to detect the patient who is more liable to suffer allergic reactions of these types.

SUMMARY

Some current views on the immunochemical mechanism of penicillin hypersensitivity are presented. The antigenicity of benzylpenicillin appears to depend in large part upon its ability to rearrange *in vivo* to its highly reactive isomer D-benzylpenicillenic acid. The postulated chemical pathways leading to the formation of the complete "penicillin antigens", and the identities of the haptenic determinants are presented. The administration of the simple chemical compound, benzylpenicillin, to experimental animals and to man leads to the production of antibodies of at least 3 different haptenic specificities. Some early views are given on the immune response of human beings to penicillin, on the possible immune mechanism involved in allergic reactions to penicillin, and on the present clinical applications of these studies.

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CELLULAR AND HUMORAL THEORIES OF ANAPHYLAXIS

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One of the oldest problems in anaphylaxis is whether the antigen reacts with cell-bound antibody or with antibody free in solution. Richet considered that the anaphylactic reaction starts with the union of antigen and antibody in serum, the antigen-antibody complex thus formed reacts with complement, giving rise to anaphylatoxin which causes anaphylactic shock; but this point of view became difficult to sustain after Dale's work on isolated preparations and his demonstration that free antibody protects from anaphylaxis rather causing it [4], [5].

A new turn has been given to this old controversy by recent findings with soluble antigen-antibody complexes.

Effects of Soluble Complexes.

It has long been known that antigen-antibody complexes can be solubilized by the addition of excess antigen. More recently it has been shown that when soluble complexes prepared *in vitro* are injected intravenously into guinea pigs they cause a syndrome resembling anaphylactic shock [7] and intracutaneously a condition resembling passive cutaneous anaphylaxis [11]. The injection of soluble complexes into the perfusate of the isolated guinea pig lung produces a bronchoconstriction and a release of histamine and "slow reacting substance" [2], [8]. These effects are similar to those of antigen in a sensitized lung. The question arises whether they are unspecific "anaphylactoid" effects or fundamentally related anaphylaxis. Some evidence on this point is provided by the competitive effect of globulins.

Competition Between Antibody and non-Antibody Globulins.

It is now well established that passive anaphylactic sensitization *in vitro* can be inhibited by non-antibody globulins [10], [17]. This effect is species-specific. Only the globulins of those species whose antibodies sensitize are capable of inhibiting passive sensitization presumably be-

cause both compete for a limited number of attachment sites on the cell. Competitive inhibition by serum globulins can thus be regarded as evidence for antibody attachment to specifiic receptors.

Inhibition of the Effects of Soluble Complexes by Globulins.

It is interesting therefore that the action of soluble complexes can also be inhibited by serum globulins. This has been demonstrated for the cutaneous reaction[12] and also for the broncho-constrictor and histamine releasing effects in the perfused lung. The globulins of non-sensitizing species e. g. bovine are not inhibitory. Presumably the same mechanism of competition for receptors operates as in anaphylactic sensitization. This implies that soluble complexes, like anaphylactic antibody, undergo cellular attachment, with the difference that the union of antigen with antibody precedes cellular fixation, whereas in anaphylaxis it follows fixation. The union of antigen with fixed antibody in the anaphylactic reaction leads to the activition of a cellular enzyme system (as explained below) but no comparable studies have as yet been carried out with soluble complexes.

Although this hypothesis is attractive it needs further experimental support. Thus it will be necessary to carry out a detailed comparison of the effects of inhibitors on the anaphylactic reaction and that of soluble complexes. It could be objected that the broncho-constrictor effects of soluble complexes occur too rapidly for cellular attachment to take place but it is now known that even passive sensitization may occur rapidly. Recent studies of passive sensitization *in vitro* have suggested that it is a two- stage process consisting of a fast reaction with low activation energy which represents attachment to receptors initially free from globulins, and a slower reaction with high activation energy required for attachment to receptors initially occupied by the animal's own gamma globulins [18]. The broncho-constrictor effects of soluble complexes in the perfused lung may involve only receptors which are initially free. This might explain the experimental findings that soluble complexes are most effective after a period of intensive preliminary perfusion with Ringer which would tend to wash away any globulins attached to the tissue [3].

The effective amounts of antibody in soluble complexes are only slightly more than those required in passive sensitization. Any difference can be explained by the fact that in passive sensitization the antibody has time to become firmly attached to the cell (possibly by multiple attachment) and hence is more effective. With regard to the function of unbound antibody it is possible that this may either induce or protect from anaphylaxis depending on whether its ratio with antigen yields soluble or insoluble complexes.

Cellular mechanisms.

By cellular mechanism of anaphylaxis I mean the cellular reactions which are initiated by the union of antigen with antibody and which lead later also to the release of histamine and other active substances. These reactions are complex and it is only recently that they have been studied systematically (see for example reviews [1], [18], [21]).

A reaction scheme proposed in conjunction with Dr. MONGAR is as follows:

The complex of antigen and cell-bound antibody reacts with a heat labile cellular constituent which in some respects resembles serum complement but is not identical with it. This reaction leads to the activation of an enzyme system which is responsible for the release of histamine and other active principles. The active enzyme system is itself short-lived and becomes inactivated within a few minutes at body temperature. At this stage the release processes initiated by the enzyme also cease. Some of the experimental findings on which this approach is based are outlined below:

1. It can be shown that the anaphylactic reaction requires a heat labile constituent present in tissues; thus if sensitized smooth muscle is heated to 44° for a few minutes and then brought back to 37° it fails to react to antigen although it is still capable of responding to histamine.

2. Several findings point to an enzymatic mechanism. Enzyme inhibitors such as iodoacetate inhibit the anaphylactic histamine release mechanism. The temperature and pH curves of histamine release correspond to typical enzyme curves. The anaphylactic mechanism is activated by calcium, a typical enzyme activator [17], [18].

3. It is possible by means of inhibitors to separate the processes of sensitization and histamine release. Thus in the presence of phenol no histamine release occurs on addition of antigen but if the phenol is subsequently removed the preparation is desensitized.

A variety of specific enzymes have been implicated in the anaphylactic reaction but will not be discussed here. I would like however to discuss briefly a problem first raised by PARROT [21] namely whether the anaphylactic reaction is an oxidative or energy requirig process.

Oxygen Consumption in Anaphylaxis.

Earlier work on the isolated guinea pig lung suggested a slight but significant increase in oxygen consumption during the anaphylactic reaction [20], [22]. It seemed possible that the smallness of this effect was due to the small proportion of cells participating in the anaphylactic process. However, in later work in which isolated sensitized rat mast cells were used, all of which might be expected to participate in the reaction, and their oxygen consumption measured by a micromanome-

tric method, addition of antigen caused no increase in oxygen consumption [13] although histamine release could be demonstrated.

Effect of Oxygen Lack.

The early finding [21] that histamine release from sensitized guinea pig lung suspended in Tyrode solution is diminished in the absence of oxygen has been confirmed [7] but the concentration of oxygen must be reduced to a very low level to obtain a substantial inhibition. In other species e. g. sensitized rat lung, oxygen lack alone does not inhibit unless glucose is also removed [6]. Addition of glucose immediately restores histamine release even in the absence of oxygen. In isolated rat mast cells a substantial reduction of histamine release can only be obtained by the combined effects of oxygen and glucose lack and the application of a thiol compound [22].

It seems obvious that we are only at the very beginning of unravelling the metabolic mechanisms involved in the anaphylactic reaction. At this stage only provisional conclusions can be drawn. The evidence so far seems to suggest that although molecular oxygen may not be a rate limiting factor nevertheless the anaphylactic reaction is basically an energy requiring process.

Release of Active Substances.

It is not proposed to discuss in detail the release of pharmacologically active substances in anaphylaxis. A feature of experiments on sensitized isolated preparations e. g. the isolated human bronchial chain [23] has been the much greater activity of antihistamines against histamine added to the bath than against the antigen. Several explanations can be proposed for this discrepancy:

1. Histamine released from mast cells impinges on smooth muscle cells in very high concentration.
2. Substances other than histamine are released.
3. The reaction of antigen with antibody attached to a smooth muscle cell may activate the contractile mechanism directly without the intervention of pharmacological agents.

Indeed, all three factors may operate together.

Conclusion

The anaphylactic reaction is being studied increasingly at a molecular level. At this level the effects of antigen-antibody complexes and classical anaphylaxis do not appear fundamentally different since

both depend on antibody fixation. Some progress has been made in the analysis of the enzymic and energetic mechanisms of the cellular anaphylactic reaction, but much still remains to be done.

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SYMPOSIUM

ALERGIA Y ENFERMEDAD ALERGICA HUMANA (E. A. H.)

THE HUMAN ALLERGIC DISEASES (H. A. D.)

ALLERGIE ET MALADIES ALLERGIQUES HUMAINES (M. A. H.)

ALLERGIE UND ALLERGISCHE ERKRANKUNG DES MENSCHEN

(A. E. M.)

B. Rose.
J. Duchaine.
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LES ANTIGENES DANS LES MALADIES ALLERGIQUES DU TYPE IMMEDIAT

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Avant d'aborder ce vaste domaine que constitue l'étude des antigènes, il est prudent de préciser le sens des mots qui seront employés.

Un antigène est toute substance qui entraîne la formation d'un anticorps spécifique dans un organisme. La signification d'"allergène" est plus restrictive mais, comme nous nous occupons ici seulement de phénomènes allergiques, on peut dire qu'ils sont synonymes. Les réactogènes sont les antigènes qui déterminent l'apparition de réagines, anticorps humoraux qui apparaissent spontanément chez les allergiques atopiques.

Si nos connaissances sur l'antigénicité ont fait récemment des progrès remarquables grâce surtout à la mise au point de nouvelles techniques immunochimiques, malheureusement, on ne peut dire que ces travaux aie déjà trouvé leur application dans la clinique allergique courante. Ce que nous savons sur la biochimie et l'immunologie des réactogènes les plus communs et sur les conditions de la sensibilisation chez l'homme est à peu près ce que nous en connaissions il y a trente ans. Un des obstacles majeurs réside toujours dans l'obligation d'expérimenter sur l'homme et surtout, dans l'échec de toutes les tentatives entreprises jusqu'à maintenant pour analyser et purifier les extraits antigéniques le plus courants. On comprend ces difficultés lorsqu'on sait que les allergènes constituent en réalité une mozaïque complexe et qu'à cet assemblage d'antigènes doit nécessairement correspondre la multiplicité des anticorps.

La réaction cutanée de type immédiat représente le type I de la nouvelle classification de COOMBS et GELL [20]; elle est appelée "anaphylactique" et caractérise deux groupes de maladies allergiques dont l'importance clinique est inégale: les affections anaphylactiques généralisées, dont l'anticorps majeur est une précipitine qui peut être mise en évidence *in vitro* et les maladies anaphylactiques locales ou atopiques, provoquées par des anticorps, les réagines, qu'on n'a pu déceler jusqu'à ce jour, et, encore, qualitativement, que par les cutiréactions ou *in vivo* par les PRAUSNITZ-KÜSTNER.

Néanmoins, des recherches récentes pour mettre fin à cette situation semblent être pleines de promesses. NOAH [34] mesure la libération de l'histamine des leucocytes dans un sang humain contenant des réagines et mis en présence de l'allergène. MILLMAN *et al.* [33] utilise une globuline anti-humaine fluorescente et, avec l'allergène de *Lolium multiflorum,* il obtient une concordance presque parfaite entre ces épreuves et le résultat des tests cutanés. Ces recherches sont encore fort délicates mais on peut espérer que la technique étant devenue plus aisée, les allergistes seront délivrés du lourd handicap que constitue le PRAUSNITZ-KÜSTNER, épreuve peu maniable et qui n'est pas exempte de dangers (hépatite infectieuse).

Bien que quelques haptènes, que certains polysaccharides microbiens puissent donner des réactions immédiates, dont la signification est difficile à interpréter, leur étude rentre plutôt dans le cadre des antigènes à réaction retardée et nous ne l'aborderons donc pas ici.

Les allergènes atopiques ont en commun diverses propriétés qui, malheureusement, ne peuvent être précisées par la chimie, la physique ou la biologie.

Ce sont le plus souvent des protéines à l'état colloïdal ou des polysaccharides de poids moléculaire élevé et qui ne passent pas à travers une membrane de collodion. En-dessous d'un poids moléculaire de 40.000, les antigènes ont un pouvoir antigénique faible mais celui-ci augmente avec l'accroissement du poids moléculaire ou lorsque la molécule antigénique est adsorbée sur une grosse particule comme le collodion [9]. Néanmoins, les recherches récentes de MAURER [31], sur l'antigénicité des polymères synthétiques d'acides aminés, a montré que le poids moléculaire n'avait pas toute l'importance qu'on lui avait attribué.

La molécule protéinique est formée par une chaine peptidique de différents acides aminés dont le pouvoir antigénique dépend de sa formule spatiale et du nombre de groupements réactifs libres (NH_2, SH, COOH) qu'elle contient et qui se combineront avec autant de molécules de l'anticorps. En fait, les groupements NH_2 jouent un rôle mineur tandis que les groupements COOH ont une importance plus grande, comparable à celle des acides aminés [40]. Mais ce sont surtout des polysaccharides avec un petit élément polypeptide [60].

Les allergènes sont des antigènes médiocres. Il est impossible de sensibiliser artificiellement l'homme à une substance aussi commune que, par exemple, le pollen, mais on peut aisément le rendre sensible au sérum de cheval, à certains médicaments ou extraits biologiques. Comme l'a remarqué CRIEP [9], l'état hypersensible a plus de chance d'apparaître si l'antigène est une substance étrangère et inhabituelle. Néanmoins, le fait que la grande majorité des individus, malgré les occasions de se sensibiliser qui leur sont offertes à chaque instant de leur existence, ne deviennent jamais allergiques, ce fait montre l'importance d'un facteur génétique, peut-être héréditaire, comme moteur de l'allergisation.

[illegible] il, chez l'individu normal, d'une adaptation harmonieuse au [illegible] ambiant, ou les allergiques seraient-ils victimes d'une mutation [illegible] qui les doterait de la faculté de pouvoir métaboliser l'anti[illegible] de la manière précise indispensable pour provoquer la réponse im[illegible] allergique? C'est ce qui ressortirait des expériences de LE[illegible] *et al.* sur des cobayes de races impures en utilisant des conjugués [illegible] haptènes de la poly-L-lysine [26].

Il est probable que nous devons étendre cette conclusion à tous les allergènes que nous connaissons actuellement. Si leur composition chimique, si leur formule nous est actuellement inconnue dans presque tous les cas, ce fait est d'autant plus regrettable qu'il nous interdit de suivre la dégradation que subit cet antigène dans l'organisme et qui aboutit à l'élément pur qui, lui, est le véritable antigène.

Il est de tradition en allergologie de classer les allergènes selon leur provenance et la voie de pénétration dans l'organisme. Nous aurons ainsi les pneumallergènes, les trophallergènes, etc. La seule classification qui serait entièrement satisfaisante devrait être basée sur la formule chimique. Comme celles-ci sont inconnues, nous pensons que la classification ne peut avoir d'autre but que de faciliter les recherches étiologiques en dressant un catalogue des renseignements spécifiques que nous fournit l'interrogatoire de nos malades. Nous laisserons de côté ici les allergènes alimentaires dont le domaine pratiquement illimité et dont l'influence, en fait fort secondaire, ont eu comme effet d'obscurcir le problème. En fait, sauf chez les enfants, les affections dues aux trophallergènes ressortissent presque toujours aux réactions retardées. Chez les rares malades qui présentent des troubles qui suivent immédiatement l'ingestion d'un aliment (urticaire des lèvres, oedème de Quincke etc.), la relation de cause à effet est si manifeste et le remède si évident qu'il est exceptionnel qu'ils estiment devoir en plus consulter un médecin.

La classification que nous proposons et dont le but est essentiellement pragmatique, comportera donc trois classes comprenant :

1. les *allergènes de la vie courante* (poussières domestiques, poils d'animaux, pollens, moisissures etc.),
2. les *allergènes de la vie professionnelle,*
3. les *allergènes médicamenteux,* que nous réduirons ici aux seuls produits biologiques qui provoquent des réactions immédiates.

1.° *Les Allergènes de la vie courante*

A. *La poussière domestique.*

C'est certainement l' allergène le plus important, le plus répandu et, en même temps, le plus mystérieux. Il est constitué par une sub-

stance antigénique propre et non par l'addition de divers allergènes domestiques comme on le croyait jadis [19].

La poussière allergénique se forme à partir des plumes, du kapok et surtout, des déchets de coton vieillissants. Les fibres neuves de kapok; les plumes fraiches n'en contiennent pas mais elle apparait au bout de 4 à 6 mois [8]. Les déchets de coton se composent des fibres courtes car les fibres plus longues sont utilisées comme textile. Rappelons que chimiquement le coton est constitué de cellulose presque pure avec des traces de cire et de graisse. On ignore par quel processus ces substances non antigéniques vont donner naissance à un nouveau produit qui, lui, sera antigénique. On a incriminé l'action des moisissures et de certains microbes mais il n'existe aucune corrélation entre les réactions cutanées à la poussière et celles à diverses moisissures [60]. DAVIES [10] a montré que des extraits de poussières sur lesquelles avaient poussé des moisissures n'étaient pas plus allergisants que les poussières qui en étaient dépourvues.

Le facteur allergisant semble être le même dans tous les pays et dans toutes les parties du monde. Mais la poussière des habitations situées au-dessus de 1000 m. d'altitude est moins active que celle provenant des plaines [57, 36]. Une température élevée, un climat humide favorisent la production de l'allergène. Ceci explique pourquoi on peut constater des différences sensibles entre des échantillons récoltés dans diverses régions d'un même pays ou même, dans les différents quartiers d'une même ville. La poussière récoltée dans les habitations situées le long des rivières, des cours d'eau souterrains ou des égouts à ciel ouvert ou dans les maisons des malades sensibles sont les plus antigéniques [44, 30, 51].

HALE et CAWLEY [22] estimant que le vieillissement et l'humidité n'étaient pas les seuls facteurs en cause dans la production de l'allergène à partir des déchets de coton, ont soumis à extraction des échantillons récoltés à des moments variables. Ils ont constaté des différences notables entre les extraits mais, contrairement aux prévisions, les échantillons les plus âgés ne se montrèrent pas les plus actifs. Les auteurs formulèrent l'hypothèse que la dégradation de la cellulose, qui donnera naissance à l'allergène poussière, débute dès la récolte, sur le terrain même et dépend peut-être de l'action du soleil et de cet ensemble de micro-organismes (moisissures et bactéries) que les fermiers appelent *cavitome*. Ces organismes ne ressemblent en rien à ceux (moisissures allergisantes et microbes banaux) qu'on avait accusés auparavant.

Bien qu'on ignore toujours le composition chimique de la molécule porteuse du pouvoir antigénique, les travaux les plus récents semblent montrer que l'allergènes, tel que nous l'utilisons, est formé d'un mélange de divers polysaccharides et de polypeptides [21]. Il résiste à la trypsine mais est détruit par la pepsine [4]. La composition varie suivant la provenance des poussières. VANNIER et CAMPBELL [58] ont trouvé 5 % de polypeptides et 95 % de polysaccharides. Il semble bien que la molécule antigénique provienne de la fragmentation d'une molécule inactive, de

poids moléculaire élevé, probablement par hydrolyse enzymatique d'origine microbienne. Cette hypothèse est renforcée par l'expérience de LIETZE et REED [27] qui ont pu rendre antigénique un extrait inactif par hydrolyse partielle à l'hydroxyde de barium.

Jusqu'à ce jour, toutes les tentatives destinées à purifier les extraits poussières n'ont pas permis d'isoler la fraction qui seule serait dotée du pouvoir antigénique. Mêmes les extraits les plus "purs" comprennent de nombreuses fractions dont la majeure partie sont allergéniques.

Les extraits commerciaux que nous utilisons pour les traitements sont non seulement un mélange complexe d'allergènes mais ils contiennent en outre de nombreuses substances non-allergisantes qui peuvent provoquer un syndrome de type infectieux avec fièvre, douleurs rhumatoïdes, céphalées [49] et qui ressemble fort à un choc histaminique modéré. L'absence des troubles caractéristiques de l'allergie différencie ce syndrome de la réaction générale qui apparait après une injection trop concentrée de solution désensibilisante. D'ailleurs, PETERSON *et al.* [41] ont découvert dans l'extrait purifié de poussières un facteur présentant l'activité d'une endotoxine. Cette propriété expliquerait certains résultats favorables paradoxaux, obtenus en traitant avec des extraits de poussières des malades atteints de bronchite chronique ou d'asthme infectieux.

Ceci, et d'autres fait difficiles à expliquer, nous porte à croire que, pour une raison ou une autre, l'allergène "poussière" se distingue des autres substances allergisantes que nous connaissons et que cette différence ne tient pas seulement à une différence dans la formule chimique mais aussi à un mode d'action qui, probablement, n'obéit pas strictement au schéma de la classique réaction antigène-anticorps telle que nous la connaissons.

B. *Les pollens.*

La pollinose est la première en date et la plus typique des maladies qu'on a pu rattacher à un mécanisme allergique. De ce fait, le pollen des différentes espèces a été l'objet de recherches étendues dans le but d'en étudier la composition chimique et de découvrir la raison pour laquelle tel pollen se révèle sensibilisant alors qu'un autre pollen, parfois de la même espèce, se montre inactif. Jusqu'à présent, aucun de ces deux points n'a été élucidé et la réponse parait encore fort éloignée.

La raison en est que, ce que nous appelons antigène pollen, est en réalité une mozaïque d'allergènes et qu'à celle-ci doit nécessairement correspondre un faisceau d'anticorps dont la composition variera selon l'individu. Si A et si B sont tous les deux sensibilisés au pollen de la Dactyle, cela ne veut pas dire que tous deux présentent rigoureusement la même image immunologique. Parmi la multitude des allergènes qu'offre le pollen de la Dactyle, le choix de A n'a pas nécessairement été le même que celui de B. La pollinose est une maladie personnelle, individuelle, où le degré de sensibilité aux diverses fractions allergéniques

contenues dans le pollen variera d'un malade à l'autre. Ceci nous permet de comprendre le phénomène de la sensibilité croisée qui existe entre de nombreuses espèces et notamment, chez les graminées. Mozaïque antigénique probablement complète chez la plupart des espèces de cette famille mais incomplète, semble-t-il, chez *Cynodon dactylon* et *Sorghum* dont les pollens donnent peu de réactions positives chez les individus sensibilisés à tous les autres pollens des graminées [50]. Ce phénomène permet de comprendre certaines réactions positives mystérieuses qu'on rencontre parfois au cours d'un examen allergologique courant. Réaction isolée, en-dehors de tout contexte pathologique et qui ne correspond à aucune sensibilité clinique décelable. S'agit-il d'une allergie latente, d'une sensibilité croisée, d'un allergène inconnu? Disons plutôt allergie à une fraction d'un antigène dont nous connaissons grosso modo l'ensemble mais dont nous ignorons le détail.

L'analyse chimique des extraits de pollen a donné des résultats qui varient suivant les auteurs. Pour PALMSTIERNA [38], l'allergéne du *Phleum pratense* aurait un poids moléculaire supérieur à 40.000. RICHTER et SEHON [45] ont isolé un pigment et un peptide composé de huit acides aminés; le pigment ne jouerait aucun rôle antigénique. AUGUSTIN et HAYWARD [2] ont analysé les extraits de pollen de la Fléole et de la Dactyle et en ont isolé deux allergènes principaux, 10 allergènes secondaires et deux fractions qui ne sont pas antigéniques.

Mais, se demande FRANKLAND [16], ces antigènes précipitants représent-ils réellement les antigènes responsables de la sensibilité cutanée? Probablement pas, car les extraits de pollen peuvent être portés à 100° C pendant 10 minutes sans subir de diminution de leur pouvoir allergénique.

BIEBERDORF *et al.* [6] ont déterminé le contenu en acides aminés de 107 pollens par chromatographie sur papier. Le nombre de ceux-ci varie de 4 à 19 suivant les familles et même suivant les espèces d'une même famille. Il semble qu'il existe une relation directe entre la quantité d'acides aminés et le pouvoir allergénique du pollen, ce qui ne veut pas dire, bien entendu, que les acides aminés sont la cause de la pollinose.

Néanmoins, la composition chimique des pollens capables de sensibiliser ne parait pas différente de celle des pollens qui ne sont pas allergisants et notamment de ceux qui ne remplissent pas le premier postulat de Thommen. L'exemple classique en est le pollen de pin. Mais, PANZANI a publié récemment deux cas de pollinose indubitable à cet abietacée avec sensibilisation certaine à différentes variétés [37]. Ce qui, une fois de plus, nous prouve que, en allergologie, les "règles" qui nous paraissent les mieux établies doivent toujours être interprétées en tenant compte de ce facteur imprévisible que constitue l'agencement personnel des anticorps chez chaque individu.

Existe-t-il non seulement une susceptibilité individuelle mais encore une susceptibilité (ou une résistance) plus générale qui se manifesterait chez tous les individus d'une même région? On pourrait le croire en comparant les habitants de Barcelone à ceux de Londres. Ces derniers

sont très rarement sensibles au pollen du platane bien que ces arbres fournissent sur leur ville plus de pollen que toutes les graminées ensemble [10], [51].

Le pollen d'Ambrosia comprend une fraction non-dialysable formée de polysaccharides neutres et acides et dont le pouvoir antigénique est élevé [41]. L'allergène se trouve dans le cytoplasme et non dans le membrane du grain de pollen [40]; cependant, les feuilles et les tiges en renfermeraient aussi mais en beaucoup moindre quantité que le pollen [41].

MALLEY *et al.* [28] ont soumis du pollen de la Fléole à des précipitations fractionnées par le sulfate ammonique à doses variables. Ils ont recueilli deux fractions dont l'une donne une réaction positive d'hémoagglutination mais qui n'est pas cuti-réactive chez les allergiques, tandis que l'autre sensibilise la peau mais ne produit pas d'hémo agglutination. Le pollen de la Fléole contient donc des réactogènes et des allergènes précipitants. Sur le plan pratique, cette question peut nous semblé académique, mais elle pourrait ouvrir la voie à des recherches qui nous permettraient de mieux connaître les allergènes et leurs vis à vis immunitaires, les réagines.

C. *Les Moisissures.*

L'allergie aux moisissures est assez difficile à diagnostiquer. Les extraits concentrés sont irritants et les solutions pour intradermoréactions ne doivent pas dépasser la concentration de 0.1 % [40]. Il est exceptionnel qu'un malade soit sensibilisé à une seule moisissure (sauf, peut-être, à Candida albicans) mais il est impossible de savoir si ceci est dû au fait que plusieurs moisissures auraient un allergène commun ou si le malade est sensibilisé simultanément à plusieurs espèces différentes. Cependant, on a signalé une réaction croisée complète entre Alternaria et Phoma [50] et peut-être, entre Alternaria et le pollen des graminées [60].

Mieux que les tests, la notion d'une aggravation saisonnière des troubles feront penser à une allergie aux moisissures. La saison des moisissures commence à la fin du printemps; elle suit le cycle vital de la végétation et dépend donc en partie des conditions locales et des cultures dominantes.

Le nombre des espèces qui ont une réelle importance au point de vue allergique est réduit et, avec quelques variations, on les rencontre dans toutes les régions du globe [50].

En Europe, les espèces saisonnières sont: cladosporium, alternaria, hemphylium, fusarium, epicoccum (maximum en été), botrytis, pullularia (qui présente deux pointes, au printemps et en automne). Par contre, penicillium, aspergillus, mucorales, aleusima, les levures et actinomycètes ne présentent aucunes variations saisonnières.

Aux Etats-Unis, le tableau est pratiquement le même sauf qu'Alternaria est plus concentré [46].

Au point de vue clinique, il est important de distinguer les moisissures atmosphériques de celles qu'on ne rencontre qu'à l'intérieur des

habitations. Comme il faut s'y attendre, ces dernières ne sont, en général, pas saisonnières.

On connait peu de choses sur la composition chimique de l'antigène. D'après RIPE et PALMSTIERNA [47], il contiendrait des acides aminés et des glucides d'un poids moléculaire de 30 à 40.000.

D. *Les poils d'animaux.*

Les poils et squames d'animaux ne constituent pas une cause fréquente de troubles allergiques, sauf chez les professionnels. L'usage de plus en plus courant du rembourrage en caoutchouc mousse ou en fibres artificielles, celui du plastic pour les jouets, la disparition quasi totale des chevaux dans les villes, la réduction marquée du nombre des chiens et des chats urbains par suite de l'exiguïté des appartements modernes, tout cela a contribué à réduire le nombre de malades qu'il faut désensibiliser contre les allergènes d'animaux domestiques.

Les extraits spécifiques sont difficiles à purifier; ils contiennent souvent des substances irritantes non-spécifiques (surtout ceux du chat). Ceci expliquerait les divergences notables qu'on rencontre chez les divers auteurs. Le pourcentage d'allergiques aux poils d'animaux s'échelonne de 6.75 % [7] à 59 % [48].

Le lieu de production de l'allergène varie suivant l'espèce. Les squames sont plus antigéniques que les poils chez le cheval, le bétail et le chien [20]. Chez le chat, la salive dont l'animal imprègne sa fourrure semble être la source principale de l'allergène. Par contre, chez le lapin, l'antigène se trouve dans les poils comme on pouvait aisément le constater jadis lorsque des literies "en poils de lapin" étaient d'usage courant.

L'allergène peut être très spécifique: tel malade sera sensible à une race de chiens et pas à une autre [23]. Mais, comme il n'existe pas de preuve directe d'une différence d'antigénicité, il se peut que la raison s'en trouve dans le fait que certains animaux répandent plus de poils et squames que d'autres. La différence serait uniquement quantitative. Mais, il existe une sensibilité croisée entre les chats et les autres félins (tigres, lions, etc.) comme en fournissent la preuve les gens du cirque [40].

Les plumes ne sont pas allergisantes, sauf pour les professionnels de la volaille. Les oreillers rembourrés de vieilles plumes sont nocifs parce qu'ils sont riches en poussière domestique. Les extraits de jeunes plumes sont inactifs. Mais, inhalées, les fines particules de plumes peuvent provoquer des troubles par irritation mécanique.

La laine, les poils de chèvre ou de chameau, comme d'ailleurs le coton, ont perdu leur antigénicité à la suite des différentes manipulations qu'on leur a fait subir pour les adapter à la filature. La sensibilité aux produits bruts existe mais, de même que l'allergie aux animaux de laboratoire, aux fourrures, elle ne concerne que les professionnels.

L'allergie aux poils humains est un problème controversé. Voorhorst [60] a trouvé une courbe de sensibilisation aux poils humains parallèle à celle à la poussière de maison. Il est probable que celle-ci ren-

forme une quantité notable de squames et poils humains, surtout depuis que l'usage des rasoirs électriques s'est répandu. En effet, la loi du moindre effort et une certaine négligence masculine incitent les usagers à souffler sur la tête du rasoir pour le nettoyer, contribuant ainsi à répandre dans l'habitation une quantité notable d'antigène.

L'allergène est difficile à identifier; s'agit-il réellement des poils et squames de la peau ou bien des moisissures, microbes, produits sébacés qui les ont contaminés? [20].

L'allergène des poils de cheval a été analysé chimiquement par STANWORTH [58]. Il aurait un poids moléculaire de 34.000, contiendrait 9 % d'hexose avec une composition en acides aminés identique à celle de la sérum albumine de cheval cristallisée [7]. Il existerait entre le poil et le sérum de cheval soit un antigène commun, soit deux antigènes distincts mais voisins et donnant lieu à une réaction croisée.

La désensibilisation spécifique est assez difficile à mener car les extraits sont souvent mal tolérés et le contact avec l'allergène est constant et intime. On peut même penser que, sauf chez les professionnels où le problème se présente autrement, l'exclusion définitive de l'animal peut seule constituer une mesure thérapeutique efficace. De plus, le diagnostic est souvent difficile car, comme le fait remarquer SPEER, le contact direct avec l'animal n'est pas indispensable. Ainsi, un enfant sensibilisé au chat peut présenter des troubles au contact avec les vêtements d'un autre enfant à l'école et qui a caressé un chat à la maison [59].

2.° *Les allergènes de la vie professionnelle*

A première vue, il apparait que les allergènes qu'on rencontre au cours de son travail sont en nombre infini. En fait, les allergènes qui nous concernent ici, c'est-à-dire ceux qui déterminent des réactions cutanées immédiates, sont peu nombreux. Ils sont néanmoins extrêmement nocifs parce qu'ils sont formés de poussières très fines et très pénétrantes et parce que le tractus respiratoire se défend mal contre les assauts répétés des substances antigéniques. Citons la farine chez les boulangers, la poussière de moulin chez les minotiers, les poils d'animaux et les farines de tourteaux chez les cultivateurs, la gomme arabique chez les imprimeurs, la sciure des bois exotiques etc. La sensibilisation est toujours très marquée, ce qui explique la fréquence des échecs des traitements spécifiques. En fait, seul le changement de métier ou de poste permet d'améliorer la situation de façon durable.

L'allergie professionnelle est assez facile à diagnostiquer quand il s'agit du type immédiat. Chaque profession est, pour ainsi dire, abonnée à un ou deux allergènes qui ont été catalogués et sont bien connus. La maladie obéit à une logique qu'on rencontre beaucoup plus rarement dans les autres sensibilisations. La difficulté réside souvent dans le diagnostic différentiel entre troubles provoqués par une intoxication, une pneumoconiose, une histamino-libération et ceux dus réellement à

une réaction antigène-anticorps. Citons, par exemple, la toux chronique des filateurs de coton, qui serait une intoxication d'origine fongique [24], la byssinose provoquée par des poussières libératrices d'histamine locale, et enfin, "le poumon des fermiers" qui n'est pas, comme on le croyait, une allergie du type immédiat au pollen du foin ou aux moisissures mais qui serait dû, d'après les travaux de PEPYS [39], à une réaction du type retardé à un antigène spécifique du foin moisi. Che certains cultivateurs qui présentent des crises d'asthme après avoir manipulé du foin moisi; les cutiréactions sont positives aux extraits de foin moisi et négatives au pollen et aux moisissures. L'allergène est inconnu (moisissure? parasite animal?); peut-être est-il le même que celui du poumon du fermier (1).

Contrairement à ce qu'on pourrait croire, vu leur mode de vie au grand air, les cultivateurs sont fréquemment atteints d'asthme professionnel. Outre le pollen (cependant la pollinose est plus rare parmi eux que chez les habitants des villes), les poils des bestiaux, les moisissures des habitations et des granges, les allergènes le plus puissants se trouvent dans les tourteaux et aliments farineux pour bestiaux (farines de fèves, de pois, de trèfle, de céréales, déchets de graines de lin et parfois de coton ou de ricin). Certains de ces produits servent aussi à l'alimentation humaine mais le passage à travers les voies digestives suppriment entièrement le pouvoir antigénique [18], [13]. Un boulanger sensibilisé à la farine peut ingérer du pain sans inconvénient.

Il est rare de constater une allergie aux fourrures chez ceux qui les portent. Le tannage et le nettoyage des peaux, la finition des fourrures ont supprimé presque tous les éléments qui pourraient agir comme allergène. Ils en ont ajouté un seul, un colorant, l'ursol, qui atteint fréquemment les ouvriers chargés de teindre les peaux et ceux qui doivent les manipuler.

Un des allergènes les plus importants est l'acide chlorogénique qu'on rencontre dans le café vert, les graines de ricin, les oranges et, probablement dans beaucoup d'autres végétaux (graine de coton, noisettes, chataignes?). Il est probable que l'acide chlorogénique se combine aux protéines du sérum pour former un haptène qui, soumis ultérieurement à l'action enzymatique, aboutirait à une quinone [17]. MAYER [29] a montré jadis que de nombreux produits simples (amines aromatiques, colorants azoïques, certains polypeptides etc.) sont antigéniques et produisent des réactions cutanées immédiates parce qu'ils se transforment aisément en quinones par oxydation.

Le seul allergène professionnel qui semble avoir été étudié au point de vue chimique est celui contenu dans la poudre d'ipéca. BERRENS et YOUNG [3] ont montré que le principe antigénique purifié était un mélange de glycoprotéines contenant 10 % d'azote et 8.5 % de glucides combinés. Le poids moléculaire serait de 30 à 40.000.

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(1) PEPYS (*Int. Arch. All.*, 24: 375, 1964) a recemment mis en évidence le rôle allergisant de certains moisissures thermophiles qui poussent sur le foin humide et surchauffé.

3.° *Les Allergènes médicamenteux*

Les produits biologiques sont utilisés comme moyens de diagnostic ou comme médicaments. Ils ne constituent qu'une partie de ce qu'on appelle "allergie médicamenteuse" et se différencient de celle-ci par le fait qu'ils provoquent des réactions du type immédiat avec réagines et parfois précipitines faciles à mettre en évidence, tandis que l'allergie aux médicaments proprement dits (aspirine, phénacétine etc.) doit être classée parmi les phénomènes du type réactif retardé.

Sérum.

L'anaphylaxie au sérum de cheval, qui est le premier des phénomènes auxquel fut reconnue une origine allergique, est devenue rare depuis l'emploi des sérums purifiés. Néanmoins, dans les cas où il existe une sensibilité aux poils de cheval, il est indispensable d'appliquer la méthode anti-anaphylactique de Besredka (injection de petites doses toutes les 30 minutes).

Pénicilline.

La pénicilline est toujours le médicament qui provoque le plus grand nombre de réactions secondaires (du type anaphylactique ou du type maladie du sérum). D'après une statistique américaine, le pourcentage des accidents et incidents de la pénicillinothérapie aurait passé, aux USA, de 5.95 % à 9.71 % entre 1954 et 1959 [32].

L'antigène se trouve dans le noyau de la molécule et non dans une des chaines latérales [5]. Il a été identifié avec l'acide pénicillénique qui se conjuguerait avec certaines protéines sériques pour former un complexe pénicilloyl-protéine et qui donnerait des cutiréactions positives chez les allergiques à la pénicilline [11]. VAN ARSDEL a montré qu'il y avait corrélation complète entre l'hémo-agglutination à ce complexe pénicilloyl-polylysine et les réactions cutanées à celui-ci et à la pénicilline.

L'acide pénicillénique se retrouvant dans la molécule de la pénicilline G et de tous ses dérivés, on comprend que la sensibilité à l'une entraîne l'allergie à tous les autres. Si, dans certains cas, les pénicillines synthétiques semblent être mieux tolérées, cela doit être attribué au fait qu'elles sont toujours prises par ingestion et dès lors perdent une grande partie de leur pouvoir antigénique en passant par le tube digestif.

Streptomycine.

Bien qu'on en ait rapporté quelques cas, l'hypersensibilité (du type immédiat) à la streptomycine ou à la tétracycline est très rare [32].

Insuline.

Les injections d'insuline peuvent donner naissance à deux anticorps allergiques différents: 1.° les anticorps neutralisants, agglutinants, liés

à la fraction gamma-globuline ou à la jonction bêta et gamma-globuline de celle-ci [55]. Ils sont thermostables et capables de neutraliser l'insuline chez l'animal sain et sont responsables de l'insulino-résistance. 2.° Des anticorps possédant les caractères des précipitines et des réagines, thermolabiles et qui provoquent les accidents allergiques qu'on rencontre de plus en plus rarements chez les diabétiques traités à l'insuline [62]. Ces anticorps ne semblent pas être dirigés contre la molécule-même de l'insuline mais contre les impuretés protéïniques que contiennent les hormones commerciales [40]. Néanmoins, on a cité des cas d'allergie, avec cutis positives, à des insulines purifiées et recristallisées à plusieurs reprises [35].

ACTH.

Il semble bien en être de même avec l'ACTH qui provoque assez fréquemment, surtout chez les asthmatiques, des phénomènes du type allergique immédiat et dont certains peuvent être très graves. Les cutiréactions sont fortement positives avec les extraits de l'ACTH commerciale qui a provoqué les troubles. Il est à peu près certain que ceux-ci sont dûs aux impuretés protéiniques que contiennent toutes les solutions commerciales. On a pu démontrer que la négativation des tests cutanés et des P. K. était parallèle au degré de purification de l'hormone [14] et que l'ACTH synthétique n'avait provoqué aucune réaction chez quatre malades qui, auparavant, avaient présenté un très haut degré de sensibilité aux solutions commerciales [15].

- Quelles sont les voies futures que nous trace l'étude des allergènes? On découvre tous les jours de nouveaux allergènes mais il existe encore une maladie assez répandue, qui est certainement d' origine allergique et dont on ignore la cause spécifique: c'est la conjonctivite printanière. Pollen? moisissure? allergène inconnu?

L'étude des allergènes à faible poids moléculaire pourrait rénover l' allergologie. Comme le fait remarquer SHULMAN, un grand pas en avant aura été fait le jour où nous pourrions remplacer nos extraits bruts et impurs de pollens, poussières, aliments ou moisissures par des allergènes chimiques simples [52].

Et ce jour n'est peut-être pas si éloigné qu'on pouvait le craindre.

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THE ANTIBODIES OF HUMAN ALLERGIC DISEASE

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In discussing the antibodies of human allergic disease we shall devote our attention primarily to the atopic diseases. These are of particular interest since they apparently represent the response of an hereditary abnormal immune mechanism to the normal contacts of daily life, while anaphylaxis and most other immunologic phenomena are the responses of normal immune mechanisms to unusual stimuli. Correspondingly, atopic sensitization is associated with unusual antibodies. The treatment of these atopic diseases by repeated injections of antigen, not only changes the amount of pre-existing antibodies but induces the formation of others which are more closely related to the ordinary antibodies. Most of these latter can be induced by experimental injection of antigens into nonatopic volunteers and so appear to represent the response of normal immune mechanisms to this procedure.

Most of the work which will be cited deals with allergy to pollen antigens, which has received the most study since it is so common. From the theoretical standpoint the pollens are a poor choice since they are relatively weak antigens when injected into experimental animals, but particularly since the usual extracts of pollens have been shown to be complex mixtures with as many as ten different antigens capable of producing distinct precipitin lines in agar gel diffusion against rabbit antibody [2].

Skin Sensitizing Antibody. The first suggestion that the serum of atopic individuals might contain a sensitizing factor was the report of Ramírez in 1919 that a previously nonallergic patient developed asthma due to horses after a transfusion of blood from a donor affected by this disease [3]. A few years later PRAUSNITZ-KUSTNER demonstrated that the serum of an atopic patient produced local passive sensitization when injected into normal skin [4].

They noted that the serum which they used did not contain precipitins, did not fix complement or show other evidence of antibody activity by the methods then available. The factor in the serum producing this phenomenon, the skin sensitizing antibody or reagin, remains the

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distinctive evidence of antibody activity in atopic diseases and the sensitization of normal human skin remains the basic test for its presence.

The biologic properties of the reagin were reviewed at the 3rd International Congress in Paris and will not be repeated in detail. Despite its development in susceptible persons after the most casual contacts, it is not easily produced by injection of antigen. It shows a strong affinity for tissues; when injected intravenously, it disappears from the circulation within a few minutes but remains fixed in the skin and conjunctivae for weeks [16]. When fixed in the tissues, it reacts to the presence of antigen by inducing the release of histamine and other physiologically active intermediary substances. In general, the greater the amount of sensitizing antibody in the body or a passively sensitized site, the less antigen needed to produce a reaction. Unlike the blocking antibodies, the reagin does not normally pass through the placenta from the maternal to fetal circulation [17, 18].

The view that the skin sensitizing antibody is the product of an abnormal immune mechanism rather than a particular route of sensitization is supported by the reports of KUHNS and PAPPENHEIMER [19] that atopic individuals immunized with diphtheria toxoid in the ordinary manner may produce a skin sensitizing type of diphtheria antitoxin which has most of the characteristics of the skin sensitizing antibody produced in response to pollen antigens.

The common laboratory animals are not susceptible to this type of sensitization. STRAUSS [5] in 1937 demonstrated that the phenomenon could be elicited in the skin of the rhesus monkey by injecting human allergic serum but the wheals produced were less distinct than in human skin and not accompanied by erythema so that the reactions were difficult to read. LAYTON has shown that the intravenous injection of EVANS blue, as for the passive cutaneous anaphylaxis in guinea pigs, produces an intense staining of the Prausnitz-Kustner lesions [6]. This renders the method more acceptable for general use and with the growing concern about the possibility of spreading serum hepatitis it is attracting increasing attention. Presumably it parallels the sensitization of human skin but precise comparisons are not yet available.

At the same time various methods have been proposed for the in vitro detection of skin sensitizing antibody. I do not refer to demonstration of hemagglutination or other manifestations of antibody activity unrelated to passive sensitization and equally present in the sera of non-allergic persons who have been injected with pollen antigens. Most actual tests of reaginic activity have depended upon the presence of living cells as indicators of passive sensitization. KATZ and COHEN [7] demonstrated that the blood cells of hay fever patients released histamine when exposed to pollen antigen in the test tube. This is a specific immunologic reaction but the use of whole blood does not distinguish the presence of antibody in the plasma from the reactions of the cells themselves. SPAIN and STRAUSS [8] reported that normal rabbit blood cells could be sensitized by human allergic serum to release histamine in the

presence of the antigen. SHELLEY [9] has described the same phenomenon, observed not by the chemical determination of histamine but by microscopic observation of the degranulation of basophil cells. However, numerous workers who have attempted to repeat his work have reported the method to be useless, because of nonspecific reactions and difficulty in interpreting the results [10], [11], [12]. Returning to the use of human blood cells as indicators of histamine release, MIDDLETON [13] showed that normal blood cells were sensitized in vitro by the serum of allergic patients, however, the results were variable and sensitization could not be accomplished in all cases. VAN ARSDEL [14] has shown that this phenomenon can be more regularly demonstrated if the serum used is fresh or stored in a frozen state. His results suggest that this approach may yield a practical in vitro method of demonstrating and measuring the sensitizing factor in human atopic serum.

For the present the classical PRAUSNITZ-KUSTNER phenomenon affords the only reliable means of detecting and studying the skin sensitizing antibody. Precise measures of its quantity in human serum are not possible but a reasonable estimate may be made by testing progressive dilutions of the serum on an individual whose skin has been shown to react normally to injection of a known positive serum.

Response to Injections of Antigen. Treatment of the atopic patient by injections of antigen produces a 2 to 4 fold increase in the titer of skin sensitizing antibodies during the first few months of treatment [20]. However, if the injection treatment is continued perennially with adequate doses this slight initial rise is followed by a progressive drop of the antibody titer leading in the course of 5 to 10 years to almost complete desensitization [21].

The repeated injections of antigens also produce many other manifestations of antibody activity. COOKE and coworkers [22] reported the presence in the sera of treated patients of a blocking antibody which did not sensitize human skin but reacted with the same antigen as the skin sensitizing antibody. LOVELESS [23] showed that this blocking antibody could be readily demonstrated in sera of treated atopic patients after skin sensitizing activity had been destroyed by heat. The serum of treated patients was subsequently shown to agglutinate particles coated with the specific antigen. Such activity was first demonstrated with collodion particles but in recent years the more frequent use of blood cells coated with antigen has led to the use of the term "hemagglutination" for such activity [24]. LIDD and FARR [25] demonstrated that the serum of treated hay fever patients specifically bound pollen antigen labeled with I-131. More recently PRESSMAN, ARBESMAN and coworkers [26], [27] have demonstrated the binding of radioactive antigen by an immunoelectrophoretic method which permits identification of the serum proteins involved in the reaction. FISHER and CONNEL [28] demonstrated that passive cutaneous anaphylaxis could be produced in the skin of the guinea pig by serum of treated hay fever patients. By agar gel

diffusion methods, precipitins have been demonstrated in the sera of treated patients by AUGUSTIN and HAYWARD [29], FEINBERG and CLINE [30] and others. Thus the injection of pollen antigens into the human atopic patient have been shown to produce the characteristic types of antibody activity induced by other antigens, and in general these same types of antibody activity are induced in nonatopic volunteers by experimental injections of antigens.

In recent years evidence derived by the most sensitive methods of determining antibody has suggested that the distinction between the antibodies of untreated and treated atopic patients is one of degree rather than kind. In this connection it is of interest to note that as early as 1930, GYORGY, MORO and WITEBSKY [31] described complement fixation with the sera of patients allergic to egg. They reported that spontaneously allergic patients showed weak fixation of complement with a broad prozone, whereas patients who had received injections of egg white showed a more typical reaction with a relatively narrow prozone. With BOYDEN's tanned cell method [32], hemagglutination was generally demonstrable with the sera of treated but not untreated allergic patients. Use of the more sensitive BDB method [33] for coating red cells with antigen has made it possible to demonstrate hemagglutination with the sera of many or most untreated hay fever patients. While LIDD originally reported the binding of I-131 labeled ragweed antigen only by the sera of treated patients [25], the use of a purified ragweed antigen (King's Antigen E) [34] produced positive reactions with a majority of the sera of untreated patients as well [35]. REISMAN and ARBESMAN [26] and PRUZANSKY, PATTERSON and FEINBERG [36] have also found radioactive ragweed antigen to be bound by sera of both treated and untreated patients. Finally, PERELMUTTER, LEA, FREEDMAN and SEHON [37] fractionating serum by cromatography on DEAE cellulose and concentrating the eluate have reported the presence of precipitins in the sera of untreated ragweed sensitive patients. Thus the sera of untreated allergic patients have been found to show traces of most of the types of antibody formerly considered to result only from treatment by injection of antigen.

The significance of these findings is not yet entirely understood. All of these types of antibody activity can be induced in nonallergic normal volunteers by the injection of antigen and no evidence has been submitted to indicate that they contribute to the allergic state. Their presence appears to indicate that the atopic person reacts to contact with antigen by producing small amounts of normal antibodies as well as the characteristic skin sensitizing antibody. There are no reports of equally rigorous investigation of the sera of normal nonallergic individuals. However, BERNTON [38] has reported that the sera of nonallergic volunteers who had received no injections of ragweed antigen showed precipitins for ragweed and hemagglutinins for ragweed detectable by the BDB method. It appears that the sensitivity of these methods has been pushed to a point where weakly positive reactions are not valid evidence of disease.

When the same sample of serum manifests so many diverse types of antibody activity it is natural to ask how many different antibodies are present. Attempts to correlate the various types of antibody activity are not easy since many of these activities can not be measured in a truly quantitative manner. When various samples of allergic sera are studied and particularly when they are exposed to immunoadsorbents there is no constant correlation between skin sensitizing activity and the other types of activity that we have mentioned [36]. In the sera of treated patients there does appear to be a correlation between hemagglutinating antibody, blocking antibody, passive cutaneous anaphylaxis and precipitins [37], depending upon the sensitivity of the various types of test. Blocking antibody can be demonstrated by the in vivo method only in sera showing relatively high titers of hemagglutinating antibody. Sera with high titers of blocking antibody show passive cutaneous anaphylaxis in the guinea pig [28] and, when concentrated, may show precipitin lines by diffusion against antigen in agar gel.

Fractionation of Serum. Further evidence of the nature of the antibodies and their inter-relationships is afforded by various physical and chemical means of fractionating the serum, such as protein precipitants, electrophoresis, ultracentrifugation, chromatography and immunoadsorbents.

Various nonspecific precipitating agents such as zinc sulfate [39], ammonium sulfate, and other inorganic salts, alcohol [41] and organic solvents, and other agents such as Rivanol [42] produce a partial separation of the proteins of the serum. While such methods may produce an enrichment of antibody activity in certain fractions, in general it has been found that skin sensitizing activity is spread over a number of fractions so that a precise separation is rarely obtained in one step. Specific precipitins for the various proteins of the serum may also be useful. However, gamma and beta 2A globulins appear to share common antigenic groups which lessen the usefulness of specific antisera in separating them [43].

Various forms of electrophoresis, Tiselius, zone and convection electrophoresis have been applied to the fractionation of allergic sera. The blocking antibody [44] and the bulk of the hemagglutinating activity [45] have been shown to move with the slower gamma 2 fraction. Localization of the skin sensitizing antibody has been less satisfactory. Using zone electrophoresis the greatest concentration of reactivity has been intermediate between the gamma and beta globulins [46] but various investigators using other techniques have reported it in the gamma [47], the beta [48] or even the alpha [49] globulin. Immunoelectrophoresis [50], which permits a greater subdivision of the protein fractions, is not applicable to the preparative fractionation of serum but is of great value in characterizing the fractions which have been separated by other methods.

Use of the ultracentrifuge has produced somewhat similar results. The blocking and hemagglutinating antibodies are shown to be present

in the 7S fraction [51]. Most investigators have found the skin sensitizing activity to be present in fractions intermediate between the 7S and 19S components, usually in the range of 8S to 14S [52]. SEHON and coworkers [51] have reported that a portion of the skin sensitizing activity is in components of high molecular weight with sedimentation of 19S or more. AUGUSTIN and HAYWARD [29] believe that the presence of skin sensitizing activity in high molecular weight components is due to complexing of skin sensitizing antibodies with macroglobulins and that the complexes were broken down by repeated centrifugation.

Ion-exchange chromotography on DEAE cellulose was applied to allergic sera by HUMPHREY and PORTER [53] and has since been used by AUGUSTIN [29], SEHON [54] and others. The bulk of the hemagglutinating activity and blocking antibody are in the first fraction eluted, while the skin sensitizing activity is scattered in several later fractions obtained at lower pH and higher ionic strength. AUGUSTIN reported that hemagglutinating and skin sensitizing activities could be completely separated by this method. SEHON [54] found that the bulk of the hemagglutinating activity was associated with blocking antibody but that some was also associated with skin sensitizing antibodies.

All of the antibody activity of allergic sera can be adsorbed by antigens linked to suitable supporting media, such as polyaminostyrene or red cells coated with antigen [55, 39]. Under suitable conditions, however, practically all of the hemagglutinating activity can be preferentially adsorbed without noticeably affecting the skin sensitizing activity [39]. Skin sensitizing antibody can also be displaced from the immunoadsorbent by blocking antibody [37] suggesting that the blocking antibody has a greater affinity for antigen than the skin sensitizing antibody.

In the fractionation of serum by these various methods, the blocking and hemagglutinating antibodies seem to be uniformly found with the typical 7S gamma globulins. On the other hand, the skin sensitizing activity in general is spread over a number of different fractions which presumably include proteins of various molecular sizes and types. This has led to the opinion that the skin sensitizing antibody is not a single molecular species but is complexed with other proteins of the serum to form a variety of complexes of relatively high molecular weight, some of which may be dissociated by the methods of fractionation of the serum [29, 56, 57]. Since many methods of fractionating serum involve a loss of one-half or more of the skin sensitizing activity it is possible that one or more components might be completely overlooked.

None of these methods have permitted isolation of the skin sensitizing antibody in amounts and a degree of purity permitting accurate chemical and physical characterization. However, they have yielded indications that neither the typical 7S gamma globulin nor the 19S beta 2M globulin alone play a significant part in the skin sensitizing activity. Elimination of these two immunoglobulins led AUGUSTIN and HAYWARD to suspect the beta 2A globulin [58]. HEREMANS and VAERMAN [40] reported the presence of skin sensitizing activity in a serum fraction composed

primarily of beta 2A globulins but with some gamma globulin, which had been separated by precipitation with zinc sulfate. FIREMAN, VANGIER and GOODMAN [43] produced a similar skin sensitizing fraction, also containing gamma and beta 2A globulins from serum by starch electrophoresis and chromotography on DEAE cellulose. When the beta 2A globulin was removed by precipitation with a specific antiserum, the skin sensitizing activity was lost.

The view that the skin sensitizing activity is carried largely or completely by the beta 2A globulin is supported by the observation that beta 2A globulin, like skin sensitizing antibody, does not normally pass through the placenta from the maternal to the fetal circulation [illegible]. It is less easily correlated with the report of REISMAN and ARBESMAN [illegible] that radioactive ragweed antigen is variably bound by gamma, beta 2A and beta 2M globulins of the sera of untreated hay fever patients.

LOVELESS [60] has reported the presence of reaginic activity in sera in which no beta 2A globulin was demonstrable by immunoelectrophoresis. MENZEL in our laboratory has separated by the use of Rivanol a protein fraction which contains more than 50 per cent of the skin sensitizing antibody of the original serum, but contains only traces of beta 2A globulin which were detected with difficulty by immunoelectrophoresis and far too small to account for the skin sensitizing activity. (Details will be given in one of the sectional meetings.)

It is this elusive type of antibody which is characteristic of human atopic disease. It alone appears to carry the sensitivity in passive transfer, and its titer in the serum of the actively sensitized individual is closely correlated with the occurrence of symptoms and the results of treatment.

Injections of antigen into the atopic individual and also into nonatopic volunteers produce ordinary 7S gamma globulin antibodies which have the property of combining with, and in sufficient concentration, of precipitating the antigen. These are demonstrable by the techniques of hemagglutination, blocking antibody, passive cutaneous anaphylaxis and precipitin tests depending upon experimental conditions such as the amount of antibody present and whether the serum has been concentrated. There is no evidence that they contribute to the causation of atopic disease and presumably they represent the normal response of the immune mechanism to injections of such antigens. In tests with immune adsorbents they have been shown to have a greater affinity for antigen than the skin sensitizing antibodies [37]. There is considerable evidence that they exert a protective function against antigen injected into the sensitive individual by competing with the sensitizing antibody and are responsible for the tolerance of increasing doses during the usual injection treatment [20, 15]. Whether they are also effective in protecting the sensitized individual against inhaled antigen remains a disputed question. The most recent evidence suggests that they exert some such influence, but that the beneficial effects of perennial treatment over a

period of years are more closely related to changes in the amount of skin sensitizing antibody than to the presence of blocking antibodies [2].

SUMMARY

The serum of patients with atopic disease is characterized by the presence of skin sensitizing antibody which combines with normal human tissues and induces the release of histamine in the presence of the antigen. When the serum is fractionated by physical or chemical means the skin sensitizing activity is largely separated from the ordinary 7S gamma globulins and characteristically is distributed over a number of fractions. A portion appears to be associated with the beta 2A globulin.

Treatment by injections of antigens is followed by changes in the titer of skin sensitizing antibody with gradual desensitization. Such treatment also induces the formation of typical 7S gamma globulin antibodies which can be demonstrated by hemagglutination, blocking techniques, passive cutaneous anaphylaxis or precipitin tests, depending upon the amount of antibody present. These antibodies can be induced in nonallergic volunteers by injections of antigen and are not unique in atopic disease. In tests with immune absorbents these ordinary antibodies have been shown to have a greater affinity for antigen than the skin sensitizing antibodies and it is probably that they produce some protective effect in the body by competing with the sensitizing antibody for antigen.

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ANTIGENS AND ANTIBODIES IN DRUG ALLERGY

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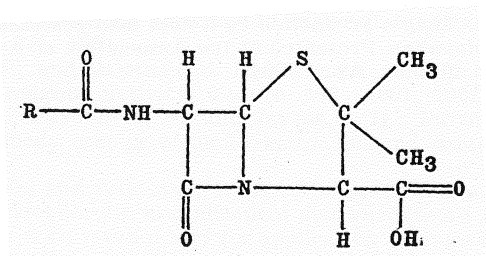
In general, drug allergy is due to antigens (or antigenic determinants) which are made, not born. Common drugs are rarely antigens per se, but require biotransformation [1] before they meet the conditions for sensitization.

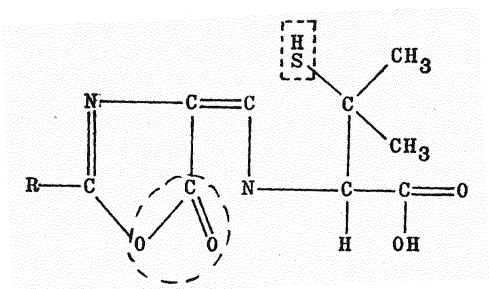
What are these conditions? It is reasonably certain that a drug, or a fragment of a drug, must become attached to a macromolecular carrier in order to induce the formation of antibodies. It is likely that a variety of macromolecular carriers will serve, but that both carrier and metabolite select each other for chemical affinities.

Labile attachment of chemicals to proteins is common, of course, and part of a physiological transport system [2], but dyes or salicylates bound to serum albumin do not sensitize. Studies on the antigenicity of penicillin suggest strongly that only chemicals with multivalent combining sites can form antigenic conjugates with proteins; that, in fact, stable univalent "haptens" might inhibit antigen antibody reactions caused by related, but multivalent antigenic determinants [3].

Penicillin:



yields a very active metabolite, penicillenic acid:



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which can form an antigenic conjugate by disulfide linkage to the half cystinyl residue, or by amide linkage to the lysyl residue of protein. The corresponding univalent hapten, —N-penicilloyl-aminocaproate, will effectively block in vitro reactions, e. g. precipitin tests for penicillin induced sensitization.

Once an antigenic compound has formed, immunologically competent cells— lymphocytes— can be expected to initiate the synthesis of antibodies. Yet, there is some uncharted territory between the formation of the antigenic conjugate and the lymphocytic response to it. It is likely that the question: "wich drugs are antigens?" and: "what type of antibodies can they be expected to induce?" must be deferred until the gap has been closed. It might be rewarding, however, to examine the nature of the gap.

It has been known for some time that elements of the reticuloendothelial system, particularly macrophages [4] participate in the sequence of events which precede antibody synthesis.

WHITE, for instance, reviewed the progress which has been made in our understanding of macrophages since SABIN described the fate of colored azoprotein in 1939 [5], but refrains from a strong commitment about their role [6]. On the basis of experiments reported by DIXON and his group [7], he seems to favor the interpretation that macrophages might transform into antibody-producing plasma cells. Actually, the answer is not known: several years ago, WISSLER, FITCH and LA VIA suggested that macrophages might be expected to form antibodies since they ingest antigen, but note that the evidence, so far, is indirect [8]; and has not dispelled the confusion which obscures the cell types which are involved in the synthesis of antibodies.

The term macrophages includes at least two classes of cells: polymorphonuclear leucocytes and mononuclear elements of the reticuloendothelial system. Once antigenic material has been admitted to the cytoplasm of the macrophage —by phagocytosis or pinocytosis— and sequestered within a membrane system its future as an antigen will depend upon its susceptibility to macrophage enzymes. LEVINE and his coworkers [9] have shown, for instance, that the capacity of randomly-bred Hartley-strain guinea pigs te become sensitized to polylysine-DNP conjugates does not depend on the "foreign-ness" of the hapten-DNP, since individual animals either became sensitive to all four hapten conjugates or to none. Thus, the immunological response might not necessarily reflect on nature of the sensitizing material, as such, but the intrinsic capacity of the animal to metabolize or not to metabolize the conjugate. Moreover, the type of macrophage which engulfs the antigen may influence the type of immunologic response since macrophages differ quantitatively in their content of hydrolytic enzymes [10].

Recently FISHMAN, in an attractive hypothesis [11] concluded that it is the macrophage which engulfs the antigenic conjugate but the immature plasma cell which synthesizes the antibody. It has been shown that antibody formation can be imitiated in vitro by innocent lymph node

cells exposed to phenol-extracted RNA obtained from macrophages which had contact with the antigen [12]. When RNA from antigen-exposed macrophages —identified by tritiated cytidine— is incubated with cells from innocent lymph nodes, lymphocytes incorporate the tritium-labelled RNA into their cytoplasm [13].

In sophisticated experiments, WEILER [14] analyzed the possible flow of information from macrophages to lymphocytes. After injection of a specific (first) antigen, he found, in the peritoneal cavity of the sensitized animals, two types of cells, (a) cells which have been "determined" by the antigen and participate in the mechanism of antibody formation; and (b) cells which are "undetermined", but immunologically competent, i.e. capable of reacting to contact with a second antigen. Transfer of this mixed population into an irradiated, isogenic recipient will produce demonstrable antibodies to the first antigen after a time lag of four days. Injection of a second antigen into the recipient will produce antibodies after a reasonable but —as expected— significantly longer interval. Incubation of the peritoneal cells, prior to transfer, with actinomycin D, will prevent the formation of antibodies to the first antigen, in the recipient animal, but will have no effect on the formation of antibodies to a second antigen, if the second antigen is injected after the transfer of the actinomycin D-incubated cells. Since actinomycin D inhibits the transfer of information from DNA to mRNA, it seems likely that the antigen-induced coding of the transferred "determined" cells has been erased; and that, consequently, antibodies fail to develop. Subsequent injection of a second antigen will produce a "normal" antibody response since the flow of information is not interrupted at a critical stage.

Studies with labelled antigens demonstrated the close structural and functional relationship between the dendritic processes of antigen containing cells and the immunologically competent cells of the germinal centers, but BRENT called attention to the fact that the difference in the rate at which antibody-producing cells appear in different species, might reflect a difference in the rate of antigen uptake by macrophages rather than a difference in the rate of antibody formation [15]. Transfer of *antigen* from macrophages into neighbouring lymphocytes has apparently not been observed.

In vivo transfer of material via a cytoplasmic bridge between the macrophage and the cells of the lymphnodes has been demonstrated by SCHOENBERG and his associates [16] who were able to show that macrophages can interact with at least two morphologically different cells in lymphoid tissue: they speculate that 19S antibodies are produced by a large mononuclear cell, presumably of the lymphocytic series, and 7S antibodies by the plasma cell.

Drugs might give rise to more than one antibody. SHULMAN [17], for instance, found in the serum of a single patient a 7S antibody which reacted only with platelets and a 19S antibody which reacted with red cells. It becomes increasingly certain that even a drug as simple as pe-

cillin does not induce the same antibody at all times: SCHWARTZ and VAUGHAN [18] have shown that at least three types of antibody may appear in penicillin-sensitive patients: 7S and 19S antibodies capable of agglutinating penicillinized red cells, and skin sensitizing antibodies that do not cause agglutination of penicillinized cells.

It should be mentioned that three of the patients studied by SCHWARTZ and VAUGHAN developed "contact dermatitis" - a fact acknowledged, but not understoodd. Some years ago, it appeared as if the "wax fraction" of the tubercle bacillus [19] was a conditio sine qua non for the development of cell-fixed antibodies, but it is now quite certain - to quote COOMBS and GELL— that "under appropriate experimental conditions virtually any antigenic material (with the possible exception of the polysaccharides)" can engender delayed sensitivity [20].

We do not know whether the "cell-fixed antibody" is really an antibody in the convential sense; and LAWRENCE prefers to call material extracted from lymphocytes which is responsible for the rejection of homografts a "factor" rather than an antibody [21]. The usefulness of delayed sensitivity in the defense of man against chemicals is still a mystery.

It might be well to conclude this review with a brief report on "work in progress", and expectations.

The number and sequence of steps which are necessary to produce drug allergy are, indeed impressive, but they do not, in principle, differ from the number and sequence of steps which lead to sensitization in general.

The *antigens* of drug allergy are, by and large, antigenic determinants, attached to protein carriers. In recent years much attention has been paid to the metabolic handling of drugs, particularly to the possible development of active metabolites, but the significance of the carrier moiety has not been clearly established.

Macrophages might dispose of conjugates of antigenic determinants and protein carrier, but their enzymatic potential is not predictable; yet, the enzymatic breakdown of the conjugate is likely to determine specificity as well as the type of the resulting antibody. In fact, concurrent stimulation of cellular activity, e.g. by Freund's adjuvant, might bring in cells of different type and, in the end, different antibodies.

The demonstration of cytoplasmic bridges which transfer "genetic information" [22] from one cell to another might solve the persistent riddle of the absence of antigen in antibody producing cells. It might also explain why sensitivity persists in spite of the limited life-span of cells which participate in the initial phase of the immunological response.

Antibodies in drug allergy are often elusive - this is not surprising since routine techniques require the ultimate antigen - which is rarely known - for a demonstration of antibodies. Even if routine techniques establish the existence of antibodies, it is often doubtful whether these antibodies are cause of effect of the disease.

Clinical differences in drug reactions are not necessarily due to differences in type of titre or antibodies: they might be due to differences in the host - response to the antigen antibody complex [23]. It is, perhaps, unfortunate that the clinical immune-response to a particular drug - e.g. the development of exfoliative dermatitis after administration of sulfonamides or p-aminosalicylic acid - might be so dramatic that symptomatic control assumes deserved priority, but leaves neither time nor inclination for basic studies. It might be possible to reverse this trend. Immunological research has advanced at a promising rate. It can be expected that the definition of the distinctive traits of an antigenic conjugate will be accomplished within a foreseeable future. Once we have clarified the relationship between such antigenic determinants and the succession of cells which participate in antibody formation our immunological assay for drug allergies might improve. At present, the application of immunological procedures to problems of drug-induced disease tends to produce results which are often surprising, generally of academic interest, but of rather limited clinical value.

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INFECTIOUS ALLERGY IN HUMAN ALLERGIC DISEASES

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The role of infection or factors thought to be supplied by infectious agents in human allergic disease has not been clear. Certainly it has not been as clearly defined as that of pollen atopy and, in terms of etiology of allergic disease, has been controversial.

Bacterial allergy, in the mechanism of common allergic syndromes, is a complex subject. The many antigenic fractions of the bacterial cell, the selective antibodies induced, the site of the antigen-antibody interaction and the relevant chemical mediators that may be involved in the production of symptoms and signs of clinical allergy are among the unsolved questions in this area.

Most studies, supporting bacterial hypersensitivity in the etiology of common allergic conditions, including asthma, vasomotor rhinitis, urticaria and angioneurotic edema, and possibly some types of allergic dermatitis, have been limited to clinical observations of closely associated acute infection with acute episodes of allergy or the demonstration of a focus of chronic infection in a chronic allergic state. There have been a number of fundamental studies, however, that add substantial emphasis to the possible relationship of bacterial hypersensitivity in the mechanism of human allergic disease.

HISTORICAL

Most of the early studies pertaining to both the delayed and the immediate type of hypersensitivity, including the initial demonstration of the tuberculin reaction by Koch [1, 2] in 1891 and the first report of anaphylaxis to bacteria, by ROSENAU and ANDERSON, in 1907, discussed below, were in the lower animal.

The delayed or tuberculin reaction was thought to be demonstrable only in an animal infected with tubercle bacili [3] until ZINSSER and PETROFF [4], using extremely large amounts of dead bacilli; and COULAND [5] incorporating solid paraffin with dead organisms; and FREUND et al. [6], using liquid paraffin established that tuberculin hypersensitivity could be induced by injection of tuberculin. Later RAFFEL [7] showed that the tu-

bacillus itself contains a lipid (wax) that, when injected with the tuberculoprotein, stimulated the development of tuberculin sensitivity. That this type of sensitivity in the lower animal is transferable by leukocytes, but not by serum, was shown by CHASE [8].

The immediate or anaphylactic type reaction to bacteria, including colon, hay, anthrax and tubercle bacilli, was first demonstrated in the guinea pig by ROSENAU and ANDERSON [9] and two years later in 1909 was shown to be transferable by serum [10]. BALDWIN [3] induced anaphylaxis but not the tuberculin reaction in the guinea pig with aqueous extract of the tubercle bacillus. ZINSSER and PARKER [11] reported anaphylaxis to typhoid antigen. Finally, ZINSSER [12] established clearly that there were two different types of tuberculin hypersensitivity, the anaphylactic and the tuberculin, the antigen of both being a protein or nucleoprotein.

In addition to nucleoprotein, some bacteria contain a carbohydrate that acts like a hapten in that it will give positive skin tests, elicit shock and contraction of the isolated uterine muscle of the guinea pig and precipitate antiserum produced by the whole bacterial substance but is not antigenic in that it will not produce antibodies. Such has been demonstrated in the pneumococcus [13], [14] as well as the tubercle bacillus [15], the hemolytic streptococcus [16], [17] and the bacillus lactis aerogenes [18] and others [19].

In man, the delayed or tuberculin type skin test has been useful in diagnosis of various bacterial diseases, including tuberculosis, brucellosis, tularemia, chancroid, and lymphogranuloma as well as fungus diseases, including histoplasmosis, coccidiomycosis and blastomycosis [20]. That this type of sensitivity in man is transferable by leukocytes but not by serum was demonstrated first by LAWRENCE [21], [22].

In man, the immediate or anaphylactic type reaction to bacteria was exemplified by the demonstration of a wheal and erythema skin test with pneumococcus polysaccharides [23] in patients with lobar pneumonia; with type-specific carbohydrates of staphylococci [24] in patients with staphylococcic infection; and specific serologic tests for group polysaccharides and the type-specific proteins of beta hemolytic streptococci [17].

Since the tuberculin-type skin reaction has been shown to be associated with particular infectious diseases, the question arises whether this delayed cellular-type of allergy may be involved in the etiology of common human allergic diseases. There is more evidence, however, to support the role of immediate type of hypersensitivity. Since the clinical symptoms and physical signs of allergic syndromes, associated with apparent infection, but not with conventional atopy as in pollinosis, are essentially the same as those obviously due to atopic factors, it would be expected that both types should be identified with the same immunologic mechanisms and chemical mediators.

Therefore, this discussion will be presented on the hypothesis that, if bacterial hypersensitivity is involved in the role of infection in human allergic disease, it should be the immediate atopic type. It is admit-

ted, however, that both the immediate and delayed type may participate under different conditions.

BACTERIAL ANTIGENS

The nature of bacterial antigens in atopic human allergic disease has not been clearly established. The sensitizing antigen most probably is a protein, as in the lower animal [9, 10, 11, 12] and the observations of KUHNS and KUHNS and PAPPENHEIMER [25, 26, 27, 28, 29, 30] with diphtheria toxoid, discussed below, supports this hypothesis. The symptom producing-antigen hay be the nucleoprotein of a carbohydrate, perhaps acting as a hapten. The exotoxins of gram-positive organisms including diphtheria and beta hemolytic streptococci are proteins [31]. The principle antigens, however, may be the polysaccharide in gram-positive organisms and a lipo-protein-polysaccharide in gram-negative bacteria [31]. The recent techniques of isolation of endotoxin from various bacteria [32, 33, 34]

TABLE I

Types of Allergic Inflammatory Responses (*)

	Early Responses		Delayed Responses
	Erythema Wheal and	Arthus or Anaphylactic	
Clinical state	Hay fever, asthma	Serum sickness	Tuberculosis, lymphogranuloma, histoplasmosis, syphilis, poison ivy
Sensitizing material	Pollens	Soluble proteins, carbohydrates	Bacteria, viruses, fungi, spirochetes, plant materials, simple chemicals
Antibody	Present in serum Non - precipitable Heat-labile	Present in serum Precipitable Heat-stable	Absent in serum Absent in cells Unknown
Transfer of sensitivity	With serum	With serum	Not with serum With cells
Cytotoxicity of antigen for explanted sensitive cells	None	None	Present

(*) From LAWRENCE, H. S. [22].

may lead to classification of the provocative antigens from these organisms.

Antibody response

The allergic inflammatory responses to various stimuli, including infection, may be divided into two types, i. e. 1st the early response, comprised of (a) the wheal and erythema variety (atopy) characteristic of hay fever in man and (b) the anaphylactic or arthus phenomenon, including serum sickness in wich precipitins are demonstrable and sensitivity is transferable by serum, and 2nd the delayed responses, usually present in infectious diseases, previously described, in which sensitivity is transferable by cells and not serum. These various responses are illustrated in Table I, taken from LAWRENCE [22].

Whereas reports have appeared of the association of the precipitating or hemagglutinating type [36] of antibody with bacterial antigens in allergic diseases (1,b above), more convincing evidence is that supporting the mechanism of atopy (1,a above).

The demonstration of wheal and erythema skin tests with antigens of pneumococcus [23] and staphylococcus [24] in nonatopic syndromes have been mentioned previously. Positive immediate and delayed skin test were demonstrated in asthmatic patients to nucleoproteins of several respiratory bacteria, both gram-positive and gram-negative [37], [38] and further evidence of the atopic type of hypersensitivity in man have been presented [39], [40], [41]. The demonstration, by SWINEFORD and HOLMAN [42] of skin-sensitizing antibodies to the polysaccharides and nucleoproteins of common respiratory organisms with sera in various allergic conditions including asthma supports strongly the role of atopy in human allergic disease.

The subject of bacterial atopy can be exemplified by two experimental and clinical models: (1) the diphtheria model and (2) the asthma model.

THE DIPHTHERIA TOXOID MODEL

In a series of experiments in man by KUHNS and PAPPENHEIMER [25], [26] and KUHNS [27] it was observed that non-precipitating, skin-sensitizing antibodies could be induced by injections of diphtheria toxoid and that the incidence of those developing such antibodies was greatest in the atopic individual with hay fever or asthma [25]. The sera, containing only non-precipitating antitoxin, resembled atopic "reagin-containing" sera in that it was capable of sensitizing normal human skin to toxin or toxoid; it remained at the injected site for many weeks; it did not fix complement, unless large quantities were used; it could be specifically co-precipitable by addition of precipitating toxin and antitoxin; and heating to 56° C for 4 hours destroyed its skin sensitizing properties,

without destroying its antitoxic effect. On the other hand, it was capable of passively sensitizing guinea pigs to fatal anaphylactic shock [26].

Furthermore, heating the non-precipitating, skin-sensitizing antitoxin to 56° C for 4 hours converted in into a blocking-type antibody that was capable of inhibiting the wheal and erythema reaction to toxoid in sensitized skin [26]. Non-precipitating, non-skin-sensitizing antitoxin formed by some patients exhibited similar blocking properties [27].

Precipitating antitoxin formed by some was incapable of sensitizing normal skin; disappeared rapidly from the injected site; fixed complement in high titer; sensitized guinea pigs; and when mixed with toxoid, inhibited the wheal and erythema reaction induced by the toxoid in sensitive skin [26]. Many individuals immunized with toxoid develop both precipitating and non-precipitating antitoxin simultaneously [26].

In comparing the effect of repeated doses of toxoid, KUHNS reported that wheal reaction occurred more frequently and of greater degree in persons receiving repeated injections when compared with those receiving single injections. Delayed reactions occurred more often in Schick-negative persons receiving repeated doses and delayed reactions generally occurred prior to the onset of immediate wheal reactions [28]. Just recently, KUHNS, using I-131 labeled toxoid, observed a rapid elimination from injected skin sites in Schick-negative subjects, except when delayed reactions occurred, suggesting that delayed reactions assisted the development of immediate wheal reactions by slowing down the release of antigen from the site of injection [29]. Even though the investigations of KUHNS and PAPPENHEIMER and KUHNS seem quite convincing, FINGER and KABAT [43] cast some doubt as to the presence of skin-sensitizing antibody to diphtheria toxoid in non-precipitating antitoxin and suggested that such activity might be due to impurities in the toxoid.

Systemic reactions to diphtheria toxoid have been observed, manifested by asthma [25, 30, 44] and by urticaria [30, 44] in individuals who had marked immediate skin reactivity and whose sera had skin-sensitizing antibodies, with and without precipitating antitoxin. It is surprising that more such systemic reactions have not occurred in atopic subjects.

THE ASTHMA MODEL

Most of the early reports pertaining to infection and asthma had to do only with clinical association of the two and the use of bacterial vaccines in the treatment of asthma [45, 46] The extensive observations and reports of WALKER [47, 50], RACKEMANN [51, 52], THOMAS and TOUART [53], COOKE [44, 54] and others [55, 60] have supported the hypothesis of bacterial allergy, particularly in asthma. Some investigators [61, 68], however, have felt that there was no primary relationship between asthma and infection but rather that the infection is a secondary factor that may increase the severity of the asthmatic state by increasing obstruction of the bronchi as a result of the infection itself or, as stated by LOWEL [69], that

there is an increased "reactivity to non-specific factors and absence of allergy".

COOKE [11], in particular, stressed the mechanism of bacterial hypersensitivity in asthma associated with infection. He based his belief upon the high incidence of allergy in antecedents of children and young adults (not in older adults with infective asthma); the occurrence of asthma associated with infection in the same patients who also have asthma from pollen, inhalants or foods; the blood eosinophilia in asthma caused by infection only; and the induction of asthma in patients following injections of respiratory bacterial vaccine.

A. *Bacterial Atopy:* The clinical syndrome of asthma is choosen as a clinical and experimental model for discussing the role of bacterial atopy in human allergic disease. At the onset, one must realize the great complexity of the subject, even though infection at times in association with the asthmatic state may be obvious. For the sake of presentation the terminology of *atopic* and *infective* asthma will be used, the latter referring to the type of asthma that is not caused by pollen, inhalant or food sensitivity but is characteristically associated with acute or chronic respiratory infection. Even though clinically, asthma is frequently caused by combined atopic and infective factors [11, 70, 71], a description of the two types is important for sake of classification and in terms of treatment and prognosis. The characteristic of each type are outlined in Table II [72].

TABLE II

Characteristics of Atopic and infective asthma (*)

	Atopic	Infective
Genetic influence	Present	Variable
Age of onset	Usually under 35	Under 5, over 35
Associated hay fever	Frequent	None
Skin tests	Usually positive	Negative
Skin sensitizing antibody	Usually present	None
Response to antiatopic therapy	Good	None
Sputum	Eosinophils	Eosins., Bacteria & PMN. Leukocytes
Associated infection	Secondary, if any	Apparently primary
Intractable asthma	Uncommon	Common
Death	Rarely	More Frequent

(*) From HAMPTON, S. F. [72].

1. *Atopic Asthma.*

This type of asthma is characterized by a prominent genetic influence and usually the family history is "positive" for asthma, or other allergy disease, in the antecedents. The onset usually is at an early age, the large majority of cases beginning in the first two decades. Onset after the age of 35 is unusual. Seasonal hay fever frequently is associated. Atopic asthma associated characteristically with positive immediate wheal and erythema skin tests and presence, in the serum, of skin sensitizing antibodies [73] to the offending antigenic factors. This type of asthma usually is amenable to treatment, both symptomatically with drugs and specifically by elimination of the the antigen or antigens or hyposensitization with extracts thereof [74]. There may be a tendency to spontaneous remissions, especially when it first occurs in infancy [75]. Sputum examinations reveal a prominence of eosinophiles and, as a rule, show no evidence of infection. Infection of the respiratory tree is absent or, when present, is of secondary nature and usually of no particular consequence. Intractable asthma and death in this type of asthma is uncommon [76], [77], [78].

2. *Infective Asthma.*

This type of asthma is characterized by a less prominent genetic influence but the family history occasionally may be "positive" for atopic disease. The onset usually is either under age 5, when the attacks characteristically are sporadic, in association with acute febrile respiratory infection, with complete remissions between episodes; or over 35, when the asthmatic state tends to become more persistent and chronic. Seasonal hay fever commonly is not associated. Skin tests with identifiable common antigens, involved in atopic asthma, are not positive. Although skin sensitizing antibodies to antigenic fractions of respiratory bacteria have been demonstrated in some cases, as discussed later, no definite immunologic mechanism has been established as yet in this type of asthma. Infective asthma usually is not as amenable to treatment and certainly not to specific anti-atopic therapy. Sputum examinations, as a rule, reveal a prominence of pus cells and bacteria as well as eosinophiles. Infections of the respiratory tract are common, not only associated with the onset of the first attack of asthma, discussed below, but frequently are identified with acute attacks of exacerbations of the asthmatic state as precipitating causes. Attacks of this type of asthma, particularly in adults, tend to become more frequent and more severe with repeated bronchial infections and most cases of intractable asthma and deaths are of this type [76], [77], [78], [79], [80], [81], [82].

B. *Acute Infection and Asthma:* As stated by SHERMAN [83], "it is well known that the symptoms of respiratory allergies may be precipita-

led by infections of the respiratory tract". Asthma, acute and chronic, follow acute specific infections of childhood, including measles, whooping cough and scarlet fever [84]; pneumonia [85]; influenza [86] and the common cold [87].

1. *First Attack of Asthma and Acute Infection.* The first attack of asthma in many patients has been reported to be associated with acute respiratory infection [87, 88, 89], including pneumonia [85], influenza [90], measles and whooping cough [84]. The author observed, during World War II, in military personnel that the first attack of asthma occurred with primary atypical pneumonia the symptoms and signs of asthma developing as the pneumonia was clearing. FAGERBERG [89], reviewed the literature on this subject and reported that in 61.9 % of 615 patients with endogenous asthma, the first attack was precipitated by respiratory infection. On the other hand, asthma has been observed to subside at the time of acute specific infectious diseases, such as measles. This phenomenon possibly could be due to competition of the antigens responsible for the asthma and that of the specific disease [90].

Although asthma is commonly associated with obvious infectious disease at the onset, occasionally neither infection nor atopy is apparent. This suggests that there may be other selective factors, presently unknown, responsible for some cases of non-atopic asthma. Aspirin intolerance [91], often manifested by severe, and fatal asthma [92, 93], and occurring in patients, apparently of the non-atopic and frequently in what may appear to be the "purely" chronic infective variety [94, 100], in one such possible example.

C. *Chronic Infection:* The role of chronic foci of infection in the respiratory tract in the cause of asthma is controversial, and opinions have been based primarily upon clinical observations.

1. *Sinuses:* Acute suppurative sinusitis may precipitate acute asthma and chronic suppuration may influence adversely the course of chronic intrinsic asthma. Whereas some have thought that sinus infections was not a major cause in asthma [64, 101, 102], others [103, 106], particularly COOKE and GROVE [107, 108], believed that not only does sinusitis contribute to the etiology of asthma but that hyperplastic sinusitis, itself, is a manifestation of bacterial allergy [109, 110].

2. *Tonsils and Adenoids:* The question of whether infection in tonsils and adenoids may play a role in the etiology of asthma has been as controversial as that of sinusitis. Some authorities [75, 111] feel that these tissues can harbor bacterial antigens that can cause of influence asthma, particularly in children, in the same manner as suppurative sinusitis, while others [112, 113] are of the opinion that this is not the case or at least that tonsillectomy and adenoidectomy have no favorable influence on asthma.

SHERMAN and KESSLER [75], state that, in selected patients removal of tonsils and/or adenoids, if actually chronically infected, usually is beneficial to the asthmatic state regardless of the nature of the mecha

nism. Whether the favorable influence is due to removal of bacterial antigen per se or merely improvement of the general health of the patient is not certain. The author agrees with SHERMAN and KESSLER [76] in that the presence of allergy does not afford any additional risk in surgical removal of tonsils and adenoids; with SOBEL [114] that indications for tonsillectomy and adenoidectomy are essentially the same in patients with and without asthma; and with GLASER [57] that recurrence of adenoidal and lymphoid tissue in the tonsillar fossae is more common in allergic children.

3. *The Bronchial Tree:* Finally, the bronchial tree itself may be chronicly infected. Thus, a patient with a chronic bronchitis may have asthma secondary to the chronic bronchitis, regardless of whether it is due to allergy to the infectious agent per se, or some unknown mechanism. Many such patients show only nonpathogenic bacteria in the sputum and bronchoscopic aspirates, and yet have asthma, possibly as an allergic response to the bacterial antigens of the normal flora of the respiratory tract, as may be the case with recurring acute infective asthma without demonstrable pathogenic bacteria, discussed below.

D. *Bacteriology of the Respiratory Tract:* Although the mouth and oropharynx harbor "normal" non-pathogenic bacteria that are considered ubiquitous, including Neisseria, alpha and gamma streptococci, diphtheroids and coagulase-negative staphylococci, the healthy lower respiratory tract is usually found to be sterile [115, 119]. On the other hand, in the diseased state, bacteria, including the normal inhabitants of the upper respiratory tract, have been cultured from bronchoscopic swabs and aspirates [116, 117, 120, 121]. PECORA and YEGIAN [115], however, did find cultures of bronchial and pulmonary tissue, removed at thorocotomy, to be sterile much more often than bronchoscopic aspirates. MAY [118], reported that in bronchitis, Neisseria catarrhalis, streptococcus viridans and non-hemolytic streptococci were found almost universally in both purulent and mucoid sputum as a results of their extension from the upper respiratory tract in a "damaged" bronchial tree, where they "colonized".

A variety of pathogenic bacteria, particularly Hemophilus influenzae, Diplococcus pneumoniae, beta hemolytic streptococci, coagulase-positive yellow staphylococci and others have been isolated from the sputum and upper respiratory tract of patients with asthma, presumably due to infection with the particular organisms [44, 56, 60, 121-126].

Some patients have asthma, however, not due to discernible atopic factors and without bacteriologic evidence of infection in the respiratory tract, when cultures of sputum, bronchoscopic aspirates or swabs may reveal only non-pathogenic ubiquitous bacteria [44, 65, 120, 124-127]. These observations support the possible role of hypersensitivity of the host to the normal flora of "oropharyngeal commensals" as spoken of by LAURENZI et al. [117].

In a study of bacterial hypersensitivity in asthma [128] cultures of sputum of 29 patients during 82 episodes of asthma, considered to have

been precipitated by acute respiratory infection, Neisseria were present in all of the 82 specimens and the growth was moderate to heavy in 79; and although other organisms, pathogens and non-pathogens, were encountered, Neisseria had the greatest intensity of growth in the majority of cultures. Differential fermentation studies on the Neisseria isolated from 41 sputum specimen revealed the following species in 74 subcultures: N. catarrhalis-10, N. flava-11, N. flavescens-4, N. perflava-20, N. pharyngis-10, N. sicca-10, N. subflava-9. The high incidence of Neisseria and other "normal" bacteria and low incidence of pathogens in the sputum of some asthmatic patients [illegible], [illegible] is suggestive, but certainly not conclusive evidence that such ubiquitous organisms may play a role in the etiology of asthma. The reader is referred to the reviews of KORTEKANGAS [116] in Europe and ROSEBURY [illegible] in the United States for detailed discussions of the bacteriology of the upper and lower respiratory tract in health and disease.

E. *The Possible Role of Bacterial Allergy in Asthma:* The role of bacterial hypersensitivity in the mechanism of asthma is uncertain and complex. Since the symptoms of both atopic and infective asthma are essentially the same, both types, theoretically, should be identified with the same immunological mechanism and chemical mediators. Thus, since atopic asthma clearly is a manifestation of the immediate type of hypersensitivity with circulating antibody, so should infective asthma be. There is some evidence favoring such relationship. The demonstration, by SWINEFORD and HOLMAN, of immediate wheal skin reaction and skin sensitizing antibodies to soluble fractions of respiratory bacteria [illegible], the induction of anaphylactic shock and desensitization in sensitized guinea pigs with bacterial antigens [129–131] and the accidental or intentional induction of asthma in man with inhalation of various bacterial substances [132–135] lends support to the concept. The reports of Hajos of the induction of asthma with respiratory bacterial antigens [illegible], including influenza virus [86] is worthy of special attention.

This concept was strengthened by a study [136] of bacterial hypersensitivity carried out in our laboratories in which asthma was induced by aerosolization of especially prepared antigenic fractions of Neisseria catarrhalis in patients who suffered from asthma associated with acute respiratory infections. Positive immediate local wheal and erythema skin reactions were present in 12 of 14 patients (55 positive reactions in 81 tests performed with all fractions). The size of the wheal, however, was small, and as rule, did not have pseudopodia, characteristic of the typical skin reaction to pollen. On the other hand, only 3 of 21 non asthmatic control subjects showed positive skin tests. Skin sensitizing antibodies were demonstrable in the sera of 5 of the 16 patients to one or more fractions. After the patients were exposed to the antigens by aerosol and by periodic injections every one to four weeks for several years, however, there was no serological evidence of blocking antibody when measured by neutralization of skin sensitizing antibody [illegible] or in

hibition of complement fixation [138] as has been shown with ragweed pollen antigen.

Inasmuch as the clinical symptoms and signs of the two types of asthma are indistinguishable one from another, presumably there is a common chemical mediator. In atopic asthma, such a mediator appears to be released from the tissues as a result of an antigen-antibody interaction which involves skin-sensitizing antibody. The demonstration of direct counterparts with some bacteria, particularly with those recorded in asthmatic individuals, is a tempting argument for the role of bacterial allergy. More directly is the accepted role of atopy to fungi [65, 139] in the etiology of asthma, and the distinction here may concern only the order of organisms involved.

F. *Possible Site of Antigen-Antibody Interaction in Asthma:* The mechanism by which infective asthma occurs remains obscure. One question is whether or not the bacterial antigens in the bronchi combine directly with antibody in the bronchial wall or indirectly by transmission via the blood from a remote site. The latter condition seems to be present in urticaria associated with a focus of infection, such as an abcessed tooth, removal of which relieves the urticaria by eliminating the source of the bacterial antigen. Whereas there are instances where the degree of asthma improves by eradication of suppurative sinusitis [104-108], the result is not nearly as complete as that with urticaria, discussed below. It is possible, of course, the reason for failure, in some cases, is that the bronchial tree, itself, is a "focus of infection" and removal of a remote focus eliminates only one source but not all sources of antigen.

A significant finding is that of DIXON and WARREN, who showed nonbacterial antigen-antibody complexes in anaphylactic shock in the guinea pig, utilizing I-131 labeled antigens, to be in the bronchial wall [140, 141]. More recently, by means of fluorescent antibody technique, the site was found to be limited to the peribronchial vessels [142, 143]. The location of the antigen-antibody complexes in asthma in man is not certain, although VÁZQUEZ and DIXON have found human gamma globulin in the bronchial wall of asthmatic lungs [144]. The interaction of antigen and antibody should take place in infective asthma in the same manner as that of atopic asthma.

OTHER SYNDROMES OF INFECTIOUS ALLERGY AND HUMAN ALLERGIC DISEASE

Other diseases of allergy, especially urticaria and angioneurotic edema and, perhaps to a less extent, atopic dermatitis, have been attributed to infective allergy.

Numerous reports refer to foci of infection as possible causes of urticaria [41, 44, 60, 63, 145, 146, 147]. BIVINGS [148] was surprised to find the hemolytic streptococcus in only 1 culture of 19 patients with urticaria

associated with sore throats. Foci of infection such as teeth [149] have appeared, at times, to be solely responsible for the urticaria. SHELDON et al. [150] have published an excellent review on the broad subject of urticaria, in which etiology, including infection, is discussed.

The author observed one patient who had urticaria only when he had a "cold". Aerosol of the same fraction of N. catarrhalis that provoked asthma in other susceptible asthmatic patients [136], induced urticaria in this individual [151].

The role of infection in the etiology of atopic dermatitis is more controversial than that of urticaria and evidence to support such relationship is based primarily on clinical observation [44, 60, 67, 152, 153]. SHERMAN and KESSLER [75] stressed three factors that supported the association, i.e. (1) exacerbation of symptoms during periods of infection in some patients, (2) the occasional child, who, following removal of infected tonsils and adenoids, makes a dramatic and sustained recovery, and (3) an excessive dose of a vaccine suspension occasionally activates the skin process.

The induction of dermatitis by aerosolization of bacterial antigens similar to that reported by TUFT [154] with inhalant allergens would support the hypothesis of bacterial hypersensitivity as a cause of atopic dermatitis.

SUMMARY

The frequent association of infection with symptoms and signs of human allergic disease has been recognized for many years. The primary question, however, has been whether it is secondary or actually plays a role in the etiology and mechanism of production of the allergic state. Since the symptoms and physical signs of allergic disease, associated with apparent infection, are essentially the same as those obviously due to atopic factors, such as pollen and other inhalants, it should be reasonable to expect both types to be identified with the same immunologic mechanism and chemical mediators.

Bacterial atopy is exemplified by the antibody response of man to diphtheria toxoid and by certain observations in human allergic disease, particularly asthma.

Diphtheria toxoid induces, in some subjects, a non-precipitating, skin sensitizing antibody encountered in hay fever and it has been observed that the atopic individual more readily develops the antibody response.

Asthma, associated with infection, resembles conventional atopic asthma in symptomatology but differs in other characteristics, including genetic influence, age of onset, association with hay fever, presence of circulating antibodies, sputum analysis, prognosis and mortality. The demonstration of the wheal and erythema type skin reaction and skin sensitizing antibodies to soluble fractions of respiratory bacteria and particularly the induction of asthma in patients with infective asthma,

but not in control subjects, with bacterial antigens, strongly supports the role of bacterial hypersensitivity, not only to pathogenic, but also to non-pathogenic bacteria that are ubiquitous to the respiratory tract.

Other allergic diseases, such as urticaria and atopic dermatitis, have been observed to be precipitated by infectious processes, possibly on a basis of bacterial atopy similar to that of asthma.

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CALCIPHYLAXIS AND CALCERGY (*)

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During the past few years we have been interested in two types of soft-tissue calcification: calciphylaxis and calcergy. In neither of these reaction forms do we have any evidence of blood-borne antibodies or other indications of a hypersensitivity in the classical immunological sense. Yet, calciphylaxis resembles anaphylaxis in that: 1. it depends upon sensitization, followed by a latency period before an appropriate challenge can evoke the characteristic response; 2. as in anaphylaxis, the mast cells play a decisive role in certain forms of both calciphylaxis and calcergy; 3. it is possible to desensitize against some of these calciphylactic and calcergic reactions by pretreatment with the normally evocative agents [9].

Calciphylaxis is a phenomenon which can induce selective calcification in various organs. It is brought about by pretreatment with a systemic calcifying compound, e. g., parathyroid hormone or vitamin D derivatives (the "sensitizer"), followed after a time interval (the "critical period"), by an eliciting agent (the "challenger").

Calcergy is an essentially different phenomenon; it is produced without previous sensitization, by parenteral administration of so called direct calcifiers or "calcergens" [14], [15].

Curiously, most calciphylactic challengers and calcergens are metals. Through their mast-cell discharging effect, histamine liberators play an important role in the mechanism of certain calciphylactic syndromes [3], [13] and the same is true of some calcergic reactions [17]. These mast cell dependent reaction types have been designated as "*mastocalciphylaxis*" [11] and "*mastocalcergy*" [17] respectively. Here, mast-cell discharge presumably participates in the distribution of the challenging metal and of calcium phosphate to different tissues [10], [17].

The main purpose of this communication is to arrive at a systematization and classification of the many variants of calciphylaxis and calcergy that have been described up to date.

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CALCIPHYLAXIS.

The most characteristic feature of all types of calciphylaxis is that they depend upon sensitization by such systemic calcifying compounds as parathyroid hormone, vitamin D_2, vitamin D_3 or dihydrotachysterol (DHT) followed by challenge in the form of mechanical trauma or treatment with certain chemicals. Although sensitization is quite indispensable, the quality of the resulting response is not significantly influenced by the choice of the particular systemic calcifier used; both the organ distribution and type of the calcifying reaction depends primarily upon the nature of the challenger and the route of its administration. On this basis, we can distinguish four prototypes of calciphylaxis:

1) local calciphylaxis,
2) systemic calciphylaxis,
3) local mastocalciphylaxis,
4) systemic mastocalciphylaxis.

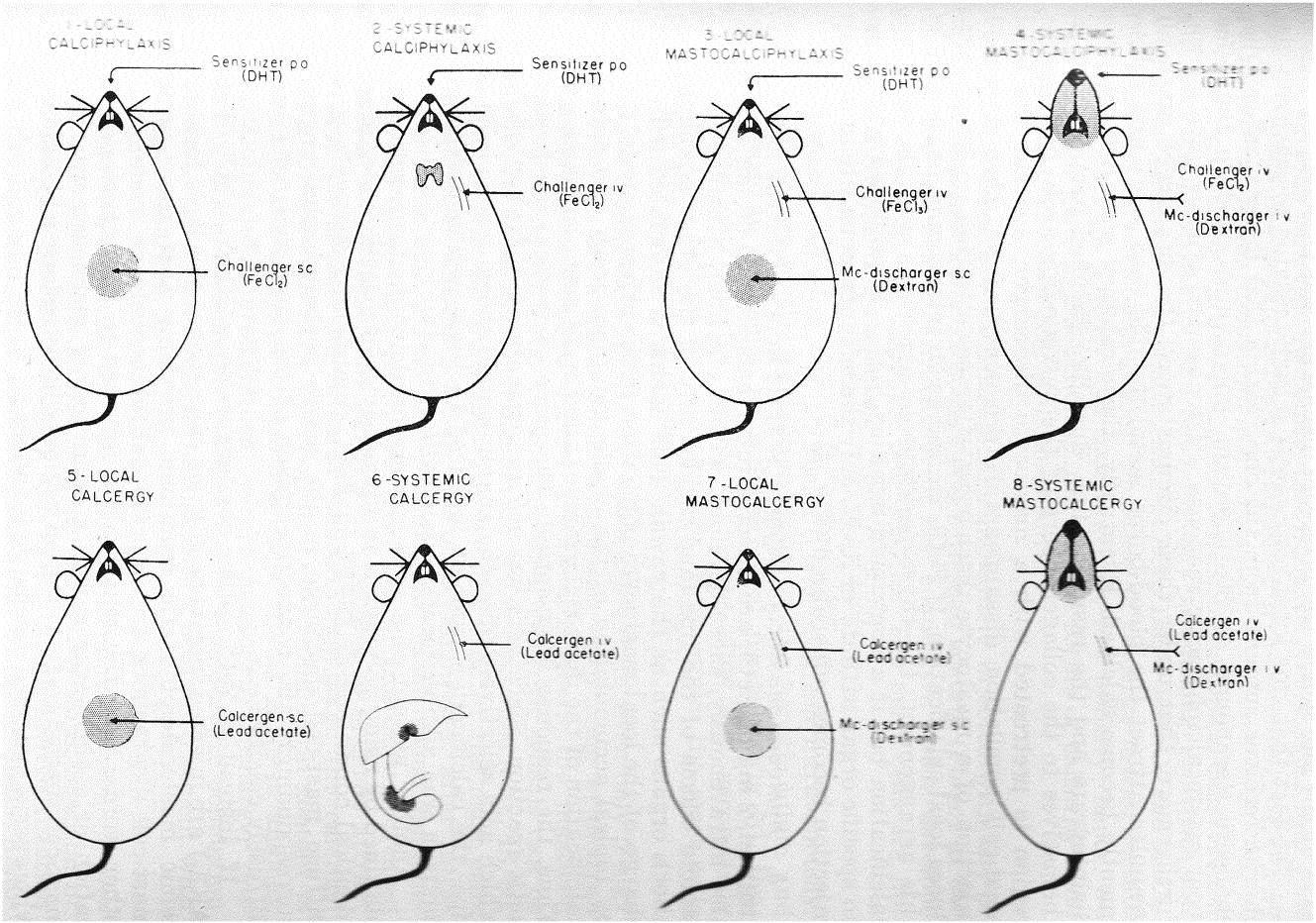
In all these forms, the sensitizer is given systemically and the reaction form is designated as local or systemic, depending upon whether the agent responsible for the localization of the lesions reaches the target area by direct topical application or through the blood stream. In simple calciphylaxis, this localizing agent is the challenger itself while, in the case of mastocalciphylaxis, a mast-cell discharger is responsible for localization, although concurrent systemic treatment with a challenger is also necessary.

We shall now try to characterize these four basic reaction forms by the description and schematic illustration of standard experimental procedures which elicit them in rats weighing approximately 100 g.

1. Local calciphylaxis.—If a rat is sensitized by the oral administration of 1 mg of DHT in 0.5 ml of corn oil by stomach tube and, after a critical period of 24 hours is challenged by the subcutaneous injection of 50 μg of $FeCl_2$, the connective tissue at the injection site becomes heavily impregnated with calcium hydroxyapatite within the next two or three days (Fig. 1). Various other challengers ($FeCl_3$, $CrCl_2$, $CrCl_3$, egg white, egg yolk or mechanical trauma to the skin) elicit a similar local calciphylactic wheal and, as we have said, the response is essentially the same if other sensitizers are employed instead of DHT.

2. Systemic calciphylaxis.—If 24 hours after the same sensitization, 1 mg of $FeCl_2$ is injected into the jugular vein, calcification occurs during the next few days in the thyroid and parathyroid glands (Fig. 2). Here, the systemically applied challenger appears to have a specific affinity for the thyro-parathyroid apparatus, and after being deposited there, secondarily attracts calcium and phosphate. Under similar conditions of sensitization, other challengers will produce more or less selective calcification in other organs depending upon their tissue affinities.

thus, ferric dextran causes calcification in the pancreas, serotonin in the salivary glands, aluminum dextran in the vagus nerves, and so forth.



3. *Local mastocalciphylaxis.*—If 24 hours after the same sensitization, 1 mg of $FeCl_2$ is injected intravenously and 2 mg of dextran subcutaneously, a large calciphylactic wheal appears after one or two days at the site where the mast-cell discharger was injected.

The fact that this local response is actually related to mast-cell discharge receives support from the observation that other mast-cell dischargers (compound 48/80, viomycin, polymyxin) produce the same local mastocalciphylactic response. Histochemical studies have shown, furthermore, that under these circumstances, the discharged mast-cell granules attract the blood-borne metal (in this case iron) and subsequently become calcified. Eventually, these calcified mast-cell granules disintegrate and the resulting fine, dust-like calcified particles attach themselves to the collagen fibers of the surrounding area. If the same region is pretreated with a mastocyte discharger, so that the local mast-cell population dissappears before sensitization, then a second injection of a mastocyte discharger after sensitization no longer produces local calcification. If $FeCl_2$ is used as an intravenous challenger in this arrangement, the thyro-parathyroid apparatus will also undergo calcification but if a compound (such as $FeCl_3$) is employed which has no specific organ affinity of itself, only the subcutaneous site of dextran injection becomes mineralized (Fig. 3).

4. *Systemic mastocalciphylaxis.*—If 24 hours after the same sensitization, 2 mg of $FeCl_2$ are injected intravenously and 60 mg of dextran also intravenously, there develops, within one hour, a marked anaphylactoid edema of the lips (and incidentally of the other anaphylactoid shock organs such as the paws). This edema is associated with a discharge of the local mastocyte population in the affected areas, followed by topical accumulation of the challenger (here iron) and subsequent calcification of the mast-cell granules and the adjacent connective tissue. Thus, the response is essentially the same as in local mastocalciphylactic reaction. Since here, we use an organotropic mast-cell discharger which acts selectively upon the anaphylactoid shock organs such as the lips, the calcification remains localized to these regions, although the dextran was administered systemically (Fig. 4).

Another variant of a systemic mastocalciphylactic reaction can be obtained by using polymyxin as a mastocyte discharger under otherwise identical conditions of DHT and $FeCl_2$ treatment. Polymyxin produces diffuse mast-cell discharge in the entire cephalo-nuchal region and correspondingly the connective tissue of the head and neck becomes diffusely calcified.

If, instead of $FeCl_2$, other challengers (e. g., $CrCl_2$, $CrCl_3$, $FeCl_3$ or $AlCl_3$) are injected intravenously, we can produce the same local or systemic mastocalciphylactic reactions, providing that the appropriate mast-cell dischargers are injected subcutaneously or systemically. In other words, here, the sensitization followed by the intravenous application of a challenger causes a condition of responsiveness in which mast-cell discharge in a limited region will be followed by calcification wherever a mast-cell discharge is induced.

CALCERGY.

The various calcergic responses can likewise be classified into four essentially similar groups except that, here, previous sensitization with a systemic calcifying compound is not required.

Calcergens are defined as substances which cause topical calcification wherever applied without the need for any sensitizing pretreatment. Among the calcergens are $PbCl_2$, $BiCl_3$, $CeCl_3$, $InCl_3$, $KMnO_4$ and $ZnCl_2$. The most convenient compound for the study of calcergy is lead acetate because of its high potency and comparatively low toxicity; hence, we shall base our general discussion of the various forms of calcergy upon experiments performed with this substance.

1. Local calcergy.—If a rat is subcutaneously injected with any of the calcergens mentioned, for example, with lead acetate (Pb-ac) at the dose of 200 μg, heavy calcification of the connective tissue develops within 24 hours at the injection site (Fig. 5). The mast cells are not visibly involved in this response.

2. Systemic calcergy.—If 10 mg of Pb-ac is given intravenously, calcification occurs in the connective tissue of the hepatic hilum and sometimes also in the cecum near the ileocecal junction (Fig. 6). Presumably, lead extravasates readily at these sites and secondarily attracts calcium, just as in local calcergy. Here again, the mast cells do not play any demonstrable role.

3. Local mastocalcergy.—If the same amount (or even only 5 mg) of Pb-ac is given intravenously, simultaneously with 2 mg of dextran subcutaneously, calcification occurs at the site where the mast cell discharger was applied (Fig. 7). Similar local calcergic wheals can be obtained by any other mast-cell discharger so far examined (e. g., 48/80, polymyxin, ovalbumin, viomycin). The participation of the mast cells in this reaction is substantiated by the observation that, after intravenous treatment with Pb-ac, lead granules can be identified histochemically in the discharged mastocyte granules of the dextran-treated subcutaneous area. These lead incrustated granules secondarily attract calcium and phosphate. As in local mastocalciphylaxis, the calcified mast-cell granules eventually disintegrate and their mineral content is transferred to the adjacent connective-tissue fibers. Also, in analogy to mastocalciphylaxis, pretreatment with a mastocyte discharger prevents this reaction, presumably because the local mastocyte population is depleted so that it can no longer respond to a second treatment with a mastocyte discharger.

4. Systemic mastocalcergy.—If, following similar intravenous pretreatment with Pb-ac, the animal is given an intravenous injection of 10 mg of dextran, there develops a calcifying anaphylactoid reaction affecting the lips and other shock organs (Fig. 8). Here again, histo-

chemical studies reveal that the calcification begins in the discharged mast-cell granules, then spreading to the surrounding connective tissue. As in all other mastocalciphylactic and mastocalcergic responses, pretreatment with mastocyte dischargers protects against this form of reaction [17].

CLINICAL IMPLICATIONS.

In view of the novelty of these concepts, little can be said as yet about their possible clinical implications. It is evident, however that in calciphylaxis and calcergy, we are dealing with fundamental reaction forms of living organisms. Numerous experiments have shown that this type of response, though primarily studied in the rat, can also be elicited in various other species, including a primate (Macacus rhesus) and hence, it may reasonably be presumed to exist in man. Indeed, there are several cases on record where hyperparathyroidism or accidental intoxication with vitamin-D compounds appears to have caused calciphylactic sensitization in patients, since they responded to trauma with local calcification [9]. In one especially interesting case of apparently nephrogenic hyperparathyroidism, cutaneous calcification was ascribed to calciphylaxis [2]. A possible calciphylactic origin has also been considered in relation to osteoma cutis [7], calcifying pancreatitis [1] and calcification in cervical intervertebral discs [6] as well as in the rapid myocardial calcification that occasionally occurs after cardiac surgery [4].

The possibility of destroying organs (and perhaps even tumors) selectively by calciphylaxis without the use of surgery also suggests possible clinical applications. Thus, it has been shown that, following suitable sensitization, appropriate challenge can cause selective calcification and loss of function in the most diverse organs: aluminum dextran in the vagus nerves [12], serotonin in the salivary glands [8], alloxan in the Langerhan's islets of the pancreas [5]. Indeed, the petrification of the thymus, induced by calciphylactic challenge with glucocorticoids, allegedly produces an immunologic incompetence which prevents the rejection of homografts [18].

It has long been suspected that there exists some relationship between disturbances of calcium metabolism and aging. In senility, the bones become brittle and there is a trend towards calcification of soft tissues (e. g., the cardiovascular system, periarticular connective tissue, crystalline lens of the eye). In rats, it has been possible to induce a progeria-like syndrome by chronic overdosage with DHT. Here, pretreatment with small doses of certain challengers induced an inverse calciphylaxis (or "anacalciphylaxis"), a condition of relative immunity to pathological calcification [16]. Curiously, this pretreatment also protected against those progeria-like changes which are not accompanied by calcium deposition (e. g., atrophy of the sex organs, thymus and skeletal musculature, loss of elasticity of the skin).

All these observations suggest certain clinical applications, but only future research will show to what extent these will assume practical importance.

In any event, the observations reported here provide us with experimental tools for the study of the long suspected interrelationship between systemic and local factors in the induction of pathologic calcification. It is common knowledge that in certain persons a local mechanical or chemical trauma can produce topical calcification and it will be interesting to explore the possible relationship between this form of abnormal reactivity and the classical forms of hypersensitivity that depend upon immunologic reactions.

SUMMARY.

An attempt has been made to present a natural classification of the various forms of calciphylaxis and calcergy. Each of these two basic prototypes is subdivided into four categories, depending upon whether the response is local or systemic and whether the mastocytes do or do not play a decisive role in its pathogenesis.

Although little is known as yet about calciphylaxis and calcergy in man, the possible clinical implications of these concepts have been briefly discussed.

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PANEL

PRUEBAS IN VITRO EN LAS E. A. H.

IN VITRO TEST IN H. A. D.

PREUVES IN VITRO DANS LES M. A. H.

IN VITRO TESTS BEI A. E. M.

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C. E. Arbesman.
R. Augustin.
A. Sehon.
E. Sorkin.

IN VITRO METHODS OF DEMONSTRATING HUMORAL ANTIBODIES OF ATOPIC INDIVIDUALS: A REVIEW (*) (**)

CARL E. ARBESMAN.

Introduction:

Following the discovery of PRAUSNITZ and KÜSTNER [1], in 1921, of the skin sensitizing antibody and the findings of LOVELESS [2], in 1940, of the blocking antibody, indicated that there were at least 2 humoral antibodies in *atopic sera*. However, until recent years these antibodies could only be demonstrated by passive transfer to non-atopic individuals. Since the development of many newer immunologic techniques in the past decade, several methods of demonstrating antibodies in vitro have been thoroughly studied.

In addition to the humoral antibodies of atopy, there are the cellular type antibodies in delayed hypersensitivity. LAWRENCE et al [3] demonstrated this antibody, and its passive sensitization by lymphocytes in non-sensitive persons. This review will consider only the immediate or humoral type of antibody in human allergic disease. The various methods of determining humoral antibodies of atopic individuals will be described very briefly. A few of these methods, with which the author has considerable experience will be dealt with in greater detail.

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METHODS OF MEASURING ANTIBODIES IN HUMAN ALLERGIC DISEASES

I. *Histamine release.*

As early as 1941, KATZ and COHEN [4] noted that the addition of ragweed extract to the whole blood of patients sensitive to this pollen, produced an increase in plasma histamine. Considerable knowledge in this phenomenon has been gained from the investigations of NOAH [5],

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MIDDLETON [6] and VAN ARSDEL [7]. Leukocytes from ragweed-sensitive donors will liberate histamine when the antigen is added. It is believed that the basophile is primarily involved. Leukocytes from non-atopic individuals can be exposed to the serum of ragweed-sensitive individuals. The addition of the ragweed antigen will cause the release of histamine. The amount released is dependent to a point on concentration of antigen added. However, beyond this optimal concentration there may be an inhibition of H-release.

Complement apparently plays no role in this reaction, but calcium is necessary. This release can be prevented by chelating agents such as EDTA, citrate and oxalate. The atopic serum tested must be fresh or stored at —70° C. [7]: frozen stored serum or heating at 56° for 45 minutes will destroy the property of sensitizing normal leukocytes.

II. *Basophile Degranulation.*

SHELLEY et al [8], of Philadelphia, have shown that when antiserum plus antigen are added to a suspension of basophiles (from rabbits) the granules will breakdown and disappear. At the American Academy of Allergy, in February 1964, reported results were rather equivocal. Spontaneous degranulation is one of the problems encountered. Temperatures and concentrations, and source of cells and concentration of antigens are all very critical.

This method of approach may prove a valuable tool in measuring antigen-antibody union if the technique can be improved and corroborated.

III. *Lymphocytes in Tissue Culture.*

Recently, Dr. HIRSHHORN [9] reported that lymphocytes obtained from sensitive patients and grown in tissue culture in the presence of antigen produce an increase in mitotic configuration showing antibody-antigen response as evidenced by growth of lymphocytes. More recently he stated [10] that observations of mitotic configuration are not necessary and merely determining the increase in ratio of large to small lymphocytes in tissue culture in the presence of the specific antigen will indicate antigen-antibody union. These techniques have been used primarily for demonstrating delayed type hypersensitivity, but it also was shown for penicillin allergy.

IV. *Radio-Active Binding.*

LIDD and FARR [11] reported that the ammonium sulphate precipitation of I-131 labelled ragweed pollen antigen and the serum of patients treated with ragweed pollen, revealed that most of these sera produced binding of the label. However, sera from untreated ragweed pollen patients did not have this property. Recently, CONNELL and LIDD [12],

using a purified fraction of ragweed pollen (Antigen E) [13], reported that about 2/3 of untreated ragweed-sensitive patients were capable of binding this antigen I-131 complex.

FRUZANSKY and PATTERSON [14], used a modification of Farr's technique, instead of Am SO_4 precipitation employed a co-precipitation method of antihuman globulin and I-131 labelled King's purified fraction "Antigen E" were able to demonstrate the binding activity in the sera

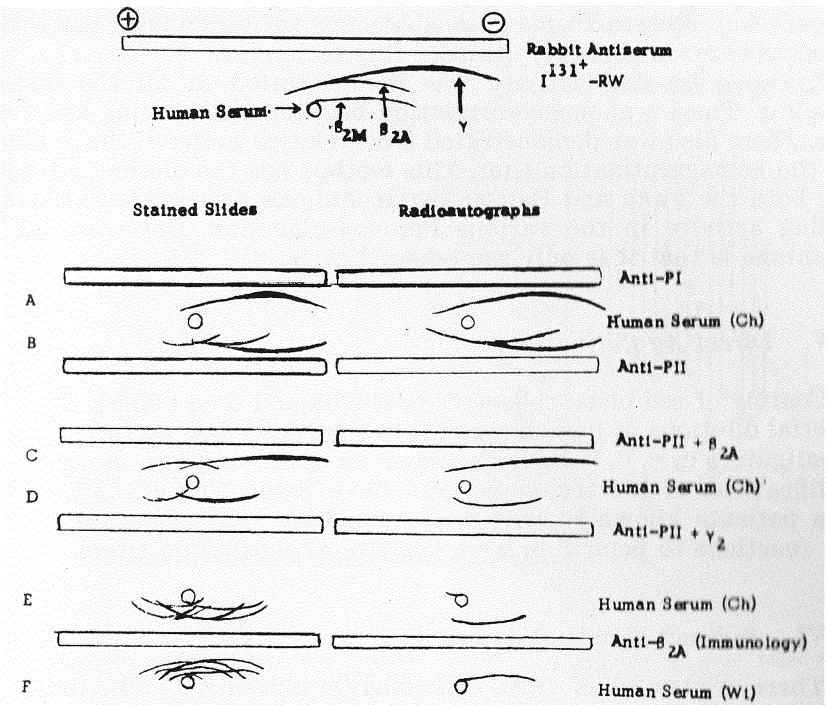

Figure 1.—Radioimmunoelectrophoresis of sera from ragweed (RW) sensitive individuals (both stained slide and radioautograph are shown. Arcs of antibody-active components of human serum are indicated on the top figure. I^{131} RW antigens were added to rabbit antiserum diffusing from the channel. Examples are shown with the sera from two sensitive individuals. Serum Ch showed the RW-binding activity in the γ-and B_{2A} globulins and Serum Wi in the γ-and B_{2M}-globulins. The vague arc in the γ_2-globulin region is the arc of complexes formed by I^{131}-RW and the precipitating antibody in the human serum. Anti-PI serum contains anti-γ antibody; Anti-PII contains anti-γ and anti B_{2A} antibody; and anti-B_{2A} serum (Immunology, Inc.) contains anti-B_{2A} and anti-B_{2M} antibody

of untreated ragweed-sensitive patients. The amount of binding, however, was greater with sera from treated patients.

YAGI et al [15] have varied both of these methods and have reported on the very sensitive procedure of radioimmunoelectrophoresis. This technique consists of:

1) The allergic patient's serum is electrophoresed. In the longitudinal well is placed a goat antiserum against either γ_2, γ_1A, or γ_{1M} human globulin. Also, in the same trough is placed I-131 labelled Pool C (a purified extract of ragweed) [16]. After 24 hours a line of precipitation develops indicating reaction between the patient's component of serum

and the particular *antiserum*. This slide is washed to remove excess antigen and stained. X-ray film is then applied to the slide and remains in contact for a period of time, depending on the radioactivity of the material used. The X-ray film developed. If there is binding of the I-131 labelled Pool C, a line caused by radiation will be noted on the X-ray film (Fig. 1)

Heating the human serum at 56° for 4 hours prior to this test will definitely diminish the γ_1A line, but does not affect the γ_2 line.

We recently reported [17] on 42 sera from untreated ragweed-sensitive patients, and 48 sera from ragweed-sensitive patients, who had specific hyposensitization therapy, utilizing this technique.

Ragweed binding activity was demonstrated in all the immuno globulins. There was some correlation between γ_1A binding and reagin titer. There also was demonstrated a correlation between the γ_2 binding and the hemagglutination titer. This method has the distinct advantage over both the FARR and PRUZANSKY techniques as it differentiates the binding activity in the various immuno-globulins. However, the disadvantage is that it is only semi-quantitative.

V. *Direct Agglutination.*

Coating of red blood cells with penicillin and then adding these cells to serial dilutions of human serum was first described by LEY [18]. Several investigators [19], [20], [21], [22] have reported on their findings using various modifications of his technique, and have found that 35-55 % of sera from patients known to have had immediate type or serum sickness-like reactions to penicillin, have positive agglutination titers.

VI. *Indirect Hemagglutination.*

There are two chief types of hemagglutination: (1) The tanned cell and (2) the B. D. B. or bis-diazotized benzidine. The B. D. B. method will be discussed by Dr. ALEC SEHON.

We have utilized the tanned cell technique primarily to demonstrate antibodies in sera of allergic patients with the following antigens:

a) *Horse serum* tanned, coated cells, when added to the sera of patients who developed serum sickness will be agglutinated by > than 1-10,000 dilution of serum. Likewise, sera in dilutions > 200 taken prior to the administration of horse serum will agglutinate these cells, if the patient is to develop serum sickness [23] (Fig. 2).

b) *Caddis Fly (Sandfly-Trycoptera)* [24] has been shown to produce positive hemagglutination titers with the sera of both treated and untreated Caddis Fly-sensitive patients.

c) *Alternaria* extract [25], only when heated at 80° C. for 5 minutes, can coat tanned red blood cells. Here, again, positive reactions are noted in the alternaria-sensitive patients. The incidence of positive reactions

and titer is greater with the sera of patients who have had specific hyposensitization therapy.

d) *Stinging Insects (Hymenoptera)* Sera from patients who have had allergic reactions from stinging insects will show positive hemagglutination titers, but they will be increased after hyposensitization therapy.

However, when one tries to coat tanned red blood cells with pure bee venom, hemolysis occurs. This difficulty has been overcome by

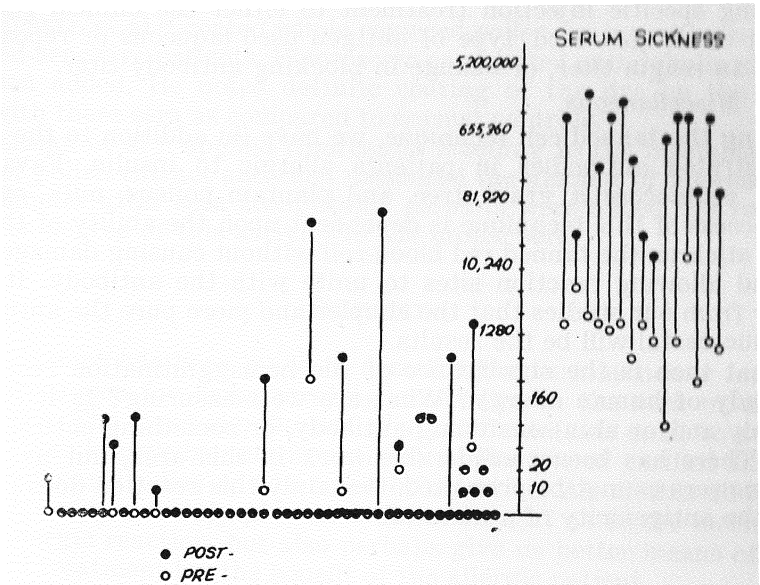

Figure 2.—Hemagglutination titers against horse serum on paired sera prior to and following prophylactic injection of 0.5 cc. of tetanus antitoxin.

using formalinized treated red blood cells prior to tanning. We have noted a much higher incidence of positive hemagglutination reactions in the sera from bee-keepers (supposedly immune to stings), than in the normal population.

c) *Ragweed Pollen and Fractions.*

1) The percentage of positive hemagglutination reactions is dependent upon the type of antigen used for coating the cells.

a) We have found that 80-90 % of sera from *untreated* ragweed sensitive patients will have positive hemagglutination reactions when a purified antigen as Goldfarb's Pool C is used [26].

b) Lower titers and less percent of positive reactions are found when whole ragweed pollen is used.

2) There is an increased hemagglutination titer following specific treatment with multiple aqueous injection treatment, repository therapy, or purified extracts [27, 30].

3) There is no increase in hemagglutination titer following Pyral ragweed injections, placebo therapy (repository or aqueous), or exposure to pollen during season.

4) Heating sera at 56° C. for 4 hours does not change the hemagglutination titer.

5) There likewise was no correlation of antibodies hemagglutination titer and degree of sensitivity of patient, age of patient, number of years patient has had symptoms, or to the reagin titer.

6) No correlation of change in hemagglutination titer (increase) following specific injection treatment to either the clinical response, amount of antigen used, type of antigen used (aqueous or repository), change in reagin titer, or change in blocking antibody titer.

f) *Miscellaneous.*

Using the tanned cell technique, we have (in addition to the above) demonstrated antibodies in patients allergic to insulin, Fava bean, ACTH, echinococcus, grass, tree, and plantain pollens, milk, egg, etc. The success of this technique is dependent upon the ability of the antigen to stick on the tanned red blood cell without causing damage to the cell and allowing reaction sites to unite with the antibody. It would appear from our studies that the simpler and more pure the antigen the more successful will be the results.

What then is the significance of the hemagglutination reaction in the study of human allergy? What are we measuring? Is it blocking antibody and/or skin-sensitizing antibody, or something entirely different? There has been considerable work in this area, but as yet the final answers cannot be presented. Certainly this reaction does demonstrate the antigenicity of a material.

VII. *P. C. A.* (*Passive Cutaneous Anaphylaxis*).

This method as described and developed by OVARY [28] has been used to demonstrate antibody in sera of allergic patients. The only successful reactions in the guinea pig were when sera from patients very intensively treated with ragweed pollen were used. This was never successful with untreated sera [29], [30]. LAYTON [31] has utilized this method, but substituting monkeys (Macacus Irus) in place of guinea pigs. Successful reactions are demonstrated with the sera of untreated sensitive patients. Hence, the skin-sensitizing antibody can fix to monkey skin. The monkey is injected with the reaginic sera intradermally. Any time after 6 hours, the antigen and Evans blue dye is injected intravenously. A positive reaction is present when a blue spot develops at the site.

The skin sensitizing antibody, using serial dilutions for sensitization, was found to be the same when titrated either in monkey or man [32].

Heating the serum at 56° C. for 2 hours (like in humans) will destroy this *reaction!* Recently, we have demonstrated and can actually determine the amount of blocking antibody in the sera of patients having received specific treatement [32].

Still another species of animal, the small white rabbit, has been used for demonstrating antibodies in the sera of patients allergic to penicillin. Cortes and HERNANDEZ [33] reported that 43 of 45 penicillin sensitive patients' sera produced positive P. C. A. when these animals were used and then challenged with penicillin intravenously.

VIII. *Fluorescein Tagging.*

CAMPBELL et al [34] coated whole ragweed pollen grains with sera from ragweed-sensitive patients. Fluorescein conjugated antiglobulin globulin was then added. He noted definite coating of the dye on the pollen grain with these sera as compared to normal controls.

MILLMAN et al [35] applied fluorescein labelling to the insoluble precipitate of pollen extract and then measured the amount of fluorescein in a fluorometer after sera from atopic patients were added. He reported a greater degree of this activity in the atopic sera.

SUMMARY

An attempt has been made to describe the various newer immunologic techniques for demonstrating antibodies in the sera of allergic individuals. The exact significance of all of these facts must be awaited with further knowledge and investigation. The use of purified antigens and specific antibodies will be most informative. All of this additional knowledge may not only give us a better insight as to the mechanism of the allergic reaction, but also perhaps give us better means of diagnosis and treatment for the benefit of the allergic patient.

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IN VITRO TESTS FOR SERUM ANTIBODIES IN HUMAN ALLERGIC DISEASES, WITH SPECIAL REFERENCE TO THE ESTIMATION OF REAGINS

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Introduction: Reagins are characteristically produced in atopic subjects through natural contact with ubiquitous substances, although this same contact does usually not cause any antibody response in non-atopic subjects; yet non-atopic subjects can also form reaginic antibodies when stimulated sufficiently strongly.

The sera of atopic subjects contain the same three classes of immune globulins [1, 2] which are found in the sera of non-atopic subjects; therefore, not surprisingly, atopic subjects form —in addition to reagins— all the various antibodies formed in non-atopic subjects. In consequence it is difficult to decide which antibodies are measured by our *in vitro* tests.

The three immunoelectrophoretically distinguishable, although structurally related, classes of immune globulins [2] are the γ_2-(= γ_{ss}), β_2A-(= γ_1A) and β_2M-(= γ_1M) globulins *. Their respective concentrations in serum are of the order of 10-14, 1-3 and 1-2 mg/ml. Although the existence of macroglobulin antibodies (β_2M) has been known for some time (e. g. isoagglutinins), β_2A-globulins were discovered immunoelectrophoretically before they were known to have antibody functions [1, 2]. Immunoelectrophoretic analyses of our highly purified reaginic fractions gave the first indication that reagins might be β_2A-globulins [3] and this was proved last year independently and simultaneously in two laboratories [4, 5]. It is still too early to say whether all β_2A-antibodies are reagins, but so far not only the reagins from ragweed [4] and grass pollen [5] sensitive subjects, but also the reagins for *Aspergillus fumigatus* [6, 7], for nematodes (Loa Loa) [7], for foreign serum proteins (serum sickness sera) [5] and for human semen [8] have proved to be β_2A-globulins. Furthermore, the physicochemical and biological behaviour of the β_2A-globulins turns out to be that of reagins [2, 5].

Before proceeding with the discussion of methods used to estimate antibodies in allergic sera, it seems advisable to review briefly in the

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* For nomenclature see [1, 2].

light of recent investigations any special properties of reagins that could be used to distinguish them from other antibodies.

Special properties of reagins re-investigated.

a) *Heat lability.* Reaginic activity is said to be destroyed by heating at 56°. However, in our experience, heating has to be continued for as long as 7 hr. to produce negative Prausnitz-Kuestner [9] (P. K.) reactions with originally positive reaginic sera [9A]. Also, while heating at 56° for only 30 min. has little effect on reaginic activity or β_2A-globulins [9A]— we found that this can sometimes greatly affect γ_2 precipitins; heated sera often give 'fuzzier' precipitation lines with antigens in agar gel than the unheated sera; precipitation titres may reduced and other alterations of the immunoelectrophoretic patterns produced [9A]. In my opinion, the effects of heating on reaginic activity are not as specific as one would expect from the literature. On the other hand, blocking activity does presumably only depend on the capacity of γ_2 antibodies to combine with allergens and this may well be a property that remains unaffected even by a partial cleavage of the antibody molecules (e. g. γ_2-globulin pieces produced by papain digestion from the parent molecule still combine with antigen although they may no longer precipitate with it [10]).

b) *Valency and binding capacity for homologous antigen.* Reagins have been shown to be absorbed by allergens coupled to insoluble supports such as polystyrenes [11, 14], red blood corpuscles [13, 13A], cow hide powder [12] and can be to some extent eluted from this combination [11, 13A]. AUGUSTIN [14] and later SEHON et al [13A] further found that these immunoabsorbents remove agglutinating antibodies more quickly than reagins from allergic sera, indicating a greater affinity between allergens and agglutinating antibodies than between allergens and reagins. MATHEWS and SPEAR [15] reported unequal absorption of reagins by red cell immunoabsorbents for which different explanations were given by AUGUSTIN [14] and by SEHON et al [13A]. Leukocytes covered with reagins take up specifically the corresponding allergens [16] (see later) * and radio-actively labelled allergens can be shown by radio immunoelectrophoresis [17] to combine with β_2A-as well as with β_2M- and γ_2-antibodies.

All this goes to show that reagins combine with the homologous allergens *in vitro* as well as *in vivo;* however, we still know nothing about the valency of reagins [14, 16]. So far, they appear to have produced neither agglutination nor precipitation reactions and may be functionally monovalent antibodies.

c) *Concentration in serum.* All estimates of the possible concentration of reagins in serum are based on their failure to give certain sensitive *in vitro* tests. However, these tests may be intrinsically incapable of measuring reagins and the reagin concentration in serum may be higher than we have been led to believe.

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* This issue "An *in vitro* test for reagins iavolving leukocytes estimation of reagins by double layer leucocyte agglutination".

d) *Sensitization of skin.* Reagins are often referred to as 'skin sensitizing antibodies' because this is one of their most outstanding properties. In this connection, sensitization means attachment to skin in such a way that interaction with the homologous antigen induces histamine release. Although presumably only antibodies with a high affinity for tissues —and in particular for mast cells— can induce histamine release during the interaction with antigen, cytophilic properties alone (as demonstrated by radioactive uptake) may not be sufficient for this effect [5], [14].

Passive cutaneous anaphylaxis (P.C.A.) in guinea pig skin is a reaction resembling P.K. tests in man, but requires the intravenous injection of dye to demonstrate histamine release due to antigen-antibody interaction. Attempts to demonstrate reagins by this method have failed [18], [19], [5] although human precipitating antisera do give the reaction [20]. On the other hand, LAYTON et al [21] found that reaginic sera give P.C.A. reactions in monkeys. It seemed essential to repeat these experiments with isolated γ_2- and β_2A-antibodies. We found [5] that β_2A-reagins gave, as expected, P.K. reactions in man and also P.C.A. reactions in monkeys, but failed to give P.C.A. reactions in guinea pigs, even when highly concentrated. On the other hand, the γ_2-antibodies prepared free from reagins from the same human sera (post-treatment pollen reaginic and serum sickness sera) gave P.C.A. reactions in guinea pigs, but not in monkeys, nor P.K. reactions in man. The P.C.A. reactions in monkeys —but not those in guinea pigs— are therefore the exact equivalent of the P.K. transfer tests in man. Incidentally, β_2M-antibodies also do not give P.C.A. reactions in guinea pigs [22]. Although the ability of β_2M-antibodies to give P.K. reactions in man and P.C.A. reactions in monkeys has not been investigated specifically, they are unlikely to give these reactions since β_2M-globulin containing fractions were found to be free from reaginic activity [4], [5], [23].

e) *Sensitization of tissues other than skin.* Reaginic sera have been found to sensitize human bronchial chains [24] and human lung [25]. We have been unable to sensitize guinea pig uteri with β_2A-reagins although this was possible with the γ_2-antibodies [26].

Currently used in vitro *tests.*

Mainly three methods are in general use; they are gel diffusion tests and direct and passive haemagglutination tests. In addition, there are the immunoelectrophoretic methods and methods in which radioactively labelled allergen preparations are added to the sera under test; any resulting complex with antibody is then precipitated either by half saturation with $(NH_4)_2SO_4$ or by rabbit antisera for human globulins. Lastly, there are tests involving histamine release due to antigen-antibody interaction.

Which antibodies are measured by these tests?

a) *Gel diffusion tests.* This is now the usual method for demonstrating precipitating antibodies. These are often found in large quantities in the sera of patients with mycetomas, e. g. of *Aspergillus fumigatus* and may occur together with reagins in the atopic type of person [29], [30].

Precipitins for moulds and vegetable dusts occur in Farmer's lung and may initiate hypersensitivity reactions of a non-reaginic type [30].

Fractionation on DEAE cellulose of a serum of an *Aspergillus* sensitive subject which contained also large amounts of precipitins, showed that the precipitins were γ_2-globulins [6] and that the reagins occurred in what is now known to be the β_2A-fraction [3], [6]. We have since confirmed that the reagins —but not the γ_2-prepitins— can be specifically precipitated by anti-β_2A-sera [7].

Since blocking antibodies occur in the γ_2-globulin fraction, sera from pollen sensitive subjects with high passive haemagglutination titres (usually present after preseasonal injection treatment) were similarly fractionated on DEAE cellulose [30]. After concentrating the isolated γ_2-globulins by pressure dialysis, precipitation lines with pollen extracts were obtained in gel diffusion tests [31] while the concentrated reaginic gave no precipitation lines, thus demonstrating once more that precipitins are γ_2-globulins. SEHON [32] later similarly isolated γ_2-precipitins from a large group of non-atopic volunteers injected with ragweed pollen extracts and we have recently produced precipitating antibodies against grass pollens *(Dactylis glomerata)* in 2/8 human volunteers [33]. Fractionation on DEAE cellulose once more showed these precipitins to be γ_2-globulins. It was further possible to show by means of gel diffusion tests and immunoelectrophoretic analyses that the precipitins thus produced were directed against the "A" antigens of grass pollens which we know *not* to be allergens [34], [13]. The human antisera did not contain antibodies for the "inner ring antigens" which we know to contain the allergens [34], [35], [13].

Serum sickness sera with precipitins for horse serum proteins were similarly fractionated and the precipitins again recovered from the γ_2-globulin fraction, with no precipitins in the isolated reaginic fraction.

Gel diffusion tests have also occasionally been positive with the the sera of drug sensitive individuals [36], [37].

I know of no case in which reaginic β_2A-antibodies have given rise to precipitin formation and it remains to be seen whether any of the precipitins found in reaginic sera are in fact formed to the same antigens that have stimulated the formation of reagins [38].

b) *Immunoelectrophoresis.* This is not strictly speaking a method for measuring antibodies; rabbit antisera for grass pollens [45], [10], moulds [6], [28], [42], [43], ragweed pollens [39] and others have been used to identify individual antigens; and so have human serum sickness sera [10], [11] and sera from mould sensitive subjects [42], [28]. There is no reason to suppose that reagins play any part in these immunoelectrophoreses.

On the other hand, by means of radio immunoelectrophoresis (to be

discussed in detail by Dr. ARBESMAN) it is possible to distinguish the particular types of antibodies that combine with a given (radioactively labelled) antigen. Not surprisingly, all three types of antibodies have been found to be involved in antibody production against mixed allergen preparations [44]. However, even if pure allergens were available, blocking antibodies as well as reagins should be found, i. e. radioactive γ_2-as well as β_2A- antigen-antibody precipitation arcs.

However, if it can be shown: 1) that the sera of all pollen allergic subjects (that give P.K. reactions) form β_2A-precipitation lines with radioactively labelled pollen extracts while the sera of non-pollen allergic subjects do not, and 2) if it can be assumed that all β_2A-antibodies are reagins, then radio immunoelectrophoresis will become a powerful tool for the demonstration of reagins. It may possibly even give some quantitative information, and the limits of sensitivity of the method will be of great interest.

c) *Indirect or passive haemagglutination tests.* This discussion is confined to our experiences with the Bis-diazotized Benzidine (B.D.B.) method [15] used for the sera of grass pollen sensitive subjects, since the situation with ragweed pollens and other antigens will be dealt with by Drs. ARBESMAN and SEHON.

Untreated grass pollen sensitive subjects gave on the whole much lower B.D.B. titres than can be found in untreated ragweed sensitive subjects [30], probably due to ragweeds being much more prolific pollinators than are grasses. Hyposensitization treatment according to NOON [46] usually resulted in an increase of B.D.B. titres, particularly when highly skin reactive subjects were selected [30]. In a later larger study [33] with unselected patients, titres remained occasionally unchanged and sometimes even decreased although there was again on the whole an increase of B.D.B. titres after the injection treatments. With P.K. titres on the whole remaining unchanged before and after treatment, there was obviously no correlation whatsoever between P.K. and B.D.B. titres. This was supported by the fact that all but a small trace of B.D.B.-antibodies were found in the $\gamma_2$globulins (free from reagins) while the isolated reaginic fractions (containing but a trace of γ_2-globulins) yielded at most a trace of B.D.B.-antibodies [30, 3]. It was concluded [39] that the B.D.B. method does not measure reagins, either because the method is instrinsically incapable of measuring reagins of because the concentration of reagins in the tested sera was below the threshold of the method [14]. Later [13], it was moreover found that the pretreatment of reaginic sera with packed red cells (the usual preliminary to B.D.B. titrations) itself removed the reagins. This and the usual heating of the sera under test for 30 min. at 56° seems certainly designed to ensure that reagins are *not* measured by this test, even though binding of reagins to the allergen-B.D.B.-cells is possible as already discussed.

d) *Methods involving radioactively labelled antigens and fluorescent antibodies* will be discussed by Dr. ARBESMAN. Again, no differentiation between reagins and other antibodies was possible and thus no correlation between the *in vitro* titres with P.K. titres.

Summarizing, none of the *in vitro* methods quoted here measure [illegible], with the possible exception of radio-immunoelectrophoresis, the antibodies measured appear to be γ_2-globulins, i. e. blocking antibodies plus probably mostly antibodies for antigens other than the allergens which are present as impurities in the allergen preparations used.

Histamine release due to antigen-antibody interaction and a recently devised test involving double layer leukocyte agglutination will be discussed in the companion paper.

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IN VITRO TESTS FOR REAGINS INVOLVING LEUKOCYTES ESTIMATION OF REAGINS BY DOUBLE LAYER LEUKOCYTE AGGLUTINATION

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In the preceeding paper it has been shown that all attempts to demonstrate reagins by precipitation, various haemagglutination reactions and radioactive binding (to which light scattering methods [1] could have been added) methods have failed, either because the physicochemical properties of reaginic β_2A-antibodies differ from those of the γ_2-antibodies or because the reagin concentration in serum is below the sensitivity of these tests. As an exception radio immunoelectrophoresis was quoted which may prove a relatively simple tool for demonstrating reagins, once certain conditions (which are pointed out) have been fulfilled. In addition it has been shown that passive cutaneous anaphylaxis [2] (P. C. A.) in monkeys is a true equivalent of the Prausnitz-Kuestner [3] (P. K.) reaction in man. We have thus an animal test capable of measuring reagins [4], but still lack an *in vitro* test.

The present paper deals with semi-biological *in vitro* tests which may be regarded as *in vitro* models of reagin-allergen reactions occurring *in vivo*.

Leukocyte damage due to antigen-antibody interaction.

It has been known for a long time that leukopenia and thrombocytopenia can occur when atopic subjects are injected with allergens. The so-called haemoclastic crisis [5] and Vaughan's leukopenic index are well known to allergists [6], [7]. There have been numerous reports that the *in vitro* addition of antigens to leukocytes (or whole blood) of sensitized animals causes immobilization [8], death [9], lysis [10-12] and/or histamine release [13-15]. The validity of these tests in respect to the estimation of reagins has been much disputed [8-18] and leukocyte damage has also been claimed for the addition of antigen to blood not containing reagins [17]. Leukocyte damage has in turn been attributed to the interaction of antigen with antibody adsorbed on the leukocyte surface [19], [20], to a mechanism resembling that of the MIDDLEBROOK-DUBOS test [21] (that is,

to the interaction of free antibody with antigen adsorbed on the leukocytes), to the secondary adsorption of antigen-antibody complexes on the leukocytes after their initial formation in the blood stream [22, 23] and to leukocytes with antibody-like properties interacting with antigen, that is to a reaction not involving serum antibodies [16, 25]. No attempt has yet been made to determine whether the antibodies involved in these reactions are γ_2-, β_2A- or β_2M-antibodies, except that WAKSMAN [12] has claimed that non-precipitating heat-stable antibodies are involved in leukocytolysis which seems to fit in best with our concept of blocking antibodies and not with that of reagins. However, valid conclusions cannot be drawn from all these rather controversial experiments.

In man, most of the blood histamine is carried by the blood basophils [26] and SHELLEY [26A] has claimed that basophil degranulation occurs when allergen is added to either human or rabbit leukocytes that have been incubated with 'allergic' sera. However, the term 'allergic', as used by SHELLEY, appears to include all sorts of drug allergies besides atopic complaints. In our hands it has been impossible to avoid spontaneous degranulation in the controls and the test has been recently severely criticized by KALLOS [27].

During 1962, I found [28] that washed human leukocytes are capable of absorbing *in vitro* reagins from grass pollen reaginic sera. Loss of activity from these sera was judged by the P. K. transfer test as well as by P. C. A. in monkeys. This absorption of reagins by leukocytes has been made the basis of the test presented here of which a preliminary report has appeared elsewhere [29].

Double layer leukocyte agglutination, and in vitro *test for reagins.*

Fig. 1 demonstrates the principle of the method. Well washed leukocytes from allergic subjects —or leukocytes from normal subjects after *in vitro* incubation with reaginic sera (3 hr. at room temperature)— are treated with excess allergen (25 min. at room temperature). This does not cause agglutination (possibly due to steric hindrance shown diagramatically as 'hypothetical prongs' in Fig. 1). However, agglutination can be produced by adding a known precipitating antiserum (species immaterial) for the allergen. The precipitating serum is thus added as an indicator, to show that allergens have been bound to the reagin-covered surface of the leukocytes. The agglutination is specific, for agglutination does not occur when either the sensitizing (reaginic) serum or the precipitating indicator serum is replaced by normal serum. Also, agglutination only occurs with homologous antigen-antibody systems, i. e. both the antisera (the sensitizing and the precipitating serum) must be specific for the same allergen; e. g. agglutination failed to occur when a reaginic serum for *Aspergillus fumigatus* was used to sensitize the leukocytes and this was followed by grass pollen allergen and rabbit antiserum for grass pollen.

We found it important to prepare the leukocytes from heparinized

cloud by sedimentation with the help of Rheo-macrodex, to avoid non specific agglutination. Further, it has been essential to wash the leukocytes thoroughly in Tyrode solution after each of the various incubations (3-5 times).

Figs. 2 and 3 show leukocyte preparations which have been treated in turn with grass pollen reaginic serum and pollen extract (containing

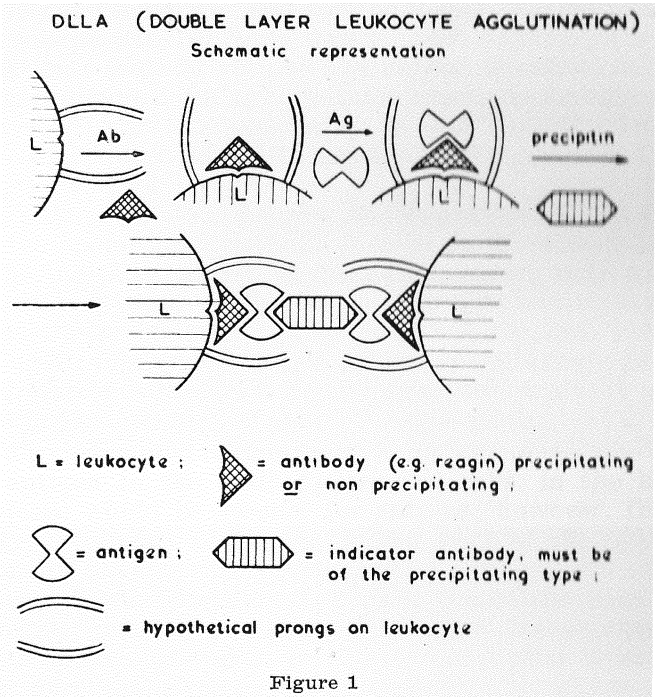

Figure 1

3000 Noon units per ml). However, the picture seen in Fig. 3 followed the addition of rabbit antipollen serum while Fig. 2 represents the control; no agglutination was obtained when normal rabbit serum was added in place of the precipitating rabbit antipollen serum. Each test series included a positive control (i. e. a known standard reaginic serum) and two negative controls; in the first negative control normal human serum was used for sensitization in place of the reaginic serum, followed by the allergen and rabbit antipollen serum and in the second negative control sensitization with the reaginic serum under test was followed by the allergen and normal rabbit serum, as described for Fig. 2.

The test was positive with reaginic sera diluted one hundredfold and was abolished when the reaginic sera were heated long enough at 56° to abolish their P.K. reactivity. The test was positive for the reaginic γ-A-fractions isolated [30] from whole reaginic sera and for reaginic sera from pollen sensitive individuals who had never been injected with allergens and whose B.D.B. titres were negative. The test thus cer-

tainly measures reagins and approaches the sensitivity of the P.K transfer reaction.

Unfortunately, the test in its present form is nevertheless not specific for reagins. Firstly, we found that heating at 56° can affect

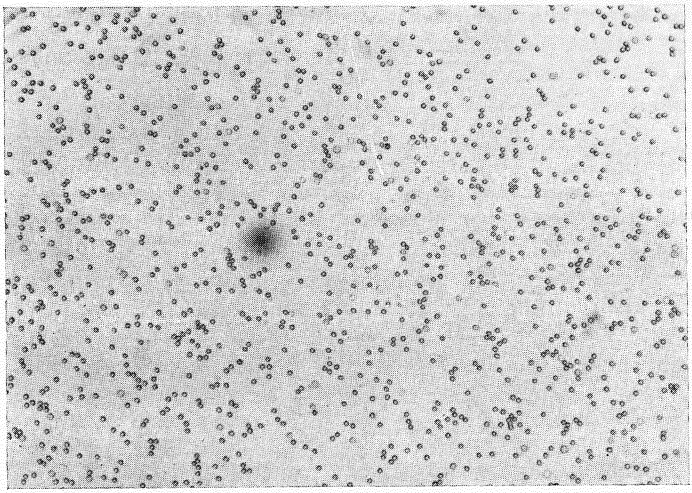

Figure 2

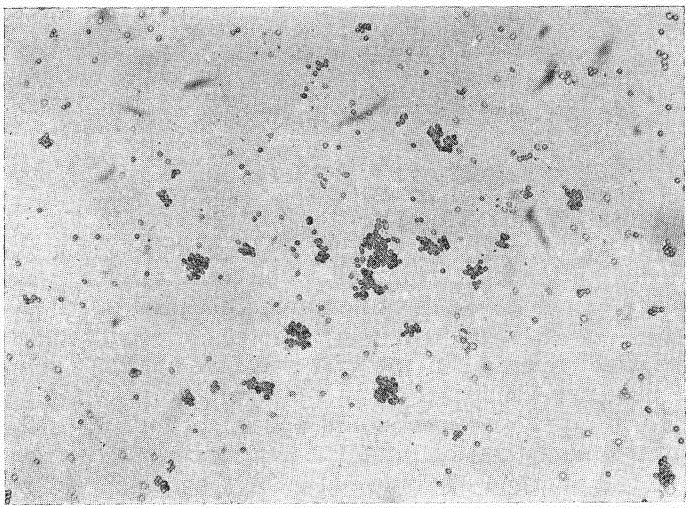

Figure 3

precipitating antibodies as well as reagins [31]. Secondly, the test was also positive when γ_2-precipitins for pollens (prepared free from reagins [30] which was checked by P. K. transfer tests) were used as the sensitizing sera in place of the reagins; thirdly, it was possible to use the γ_2-pre-

sera from serum sickness and Hashimoto sera for the sensitization normal leukocytes for horse serum and thyroglobulin antigens respectively. Positive results were also obtained with the appropriate tuberculin system (leukocytes from Mantoux positive individuals or normal leukocytes incubated with sera from Mantoux positive subjects, followed by tuberculin and guinea pig precipitins for tuberculin) [32].

The test is very sensitive, also for the demonstration of γ_2-antibodies, e.g. it was positive with the sera (and leukocytes) of siblings of Hashimoto patients (but not with sera of normal unrelated individuals) who gave negative tamed cell agglutination reactions for thyroglobulin.

Double layer leukocyte agglutination is thus capable of measuring antibodies (γ_2- *or* β_2A-antibodies) in serum as well as attached to leukocytes.

Some distinction between reagins and γ_2-antibodies has been possible. When the same (human) serum is used as the sensitizing serum as well as the indicator serum, agglutination occurs only with precipitating (γ_2-antibody) sera —and never with reaginic (β_2A antibody) sera, provided the precipitating titres were not too low. Further, we are now trying to distinguish between non-precipitating γ_2-antibodies and non-precipitating β_2A-reagins by adding a third antibody layer consisting of antibodies specific for γ_2-and β_2A-globulins.

We have also attempted to demonstrate histamine release due to the addition of antigen to the antibody covered cells, in the hope that histamine release would be specific for reagins; however, the results have been very irregular, except that so far none of the cells covered with γ_2-antibodies have released histamine on contact with the corresponding antigen. Similar irregularities have meanwhile been reported by VAN ARSDELL [33] who nevertheless claims that he can demonstrate reagins in this manner. However, he has not attempted to test specifically whether pure γ_2-antibodies can cause histamine release.

A further possibility is to pretreat reaginic serum with mercapto-ethanol which destroys reaginic activity, but does not affect γ_2-antibodies.

We are also attempting to determine whether there is not after all some difference in the affinity of reagins and γ_2-antibodies for leukocytes by measuring radioactive uptake of tagged allergens to the antibody-covered leukocytes and of tagged β_2A- and γ_2-antibodies to normal leukocytes.

Summarizing, β_2A-globulins —and thus reagins— can be distinguished from other antibodies by differences in chemical structure (demonstrable by antisera specific for the three main classes of globulins), by differences in their reactivity to mercapto-ethanol and by certain biological peculiarities. Of the latter, failure to pass the placental barrier is not of much use in practice (and is shared with β_2M globulins), but histamine release due to the interaction with antigen in the presence of suitable cells or with the reagins attached to these cells, may well prove specific for reagins, although obviously difficult to standardize.

Any specific *in vitro* test for reagins must presumably be ve sensitive as well as include some special property of reagins that distin guishes them from other antibodies. Our experiments show that hea lability and affinity for leukocytes are not amongst these special pro perties, but that the D.L.L.A. *in vitro* test presented here certainly do measure reagins with a sensitivity approaching that of the P.K. reac tion itself.

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CONTRIBUTION TO THE PANEL DISCUSSION ON "IN VITRO TESTS IN HUMAN ALLERGIC DISEASES"

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Introduction.

In spite of the very promising title of this Panel Discussion and in spite of the many painstaking investigations in numerous laboratories, it can be stated from the outset that the search for a reliable in vitro diagnostic test for human allergic diseases (H. A. D.) has so far met with little success. Amongst the many reasons for this failure one may cite that (i) none of the antibodies involved in allergic diseases has been isolated in a pure form and in consequence the nature of these antibodies has eluded a precise definition, and (ii) none of the many in vitro techniques developed for the detection of antibodies mediating allergic conditions is capable of discriminating between the different types of antibodies present in allergic sera.

It is fairly obvious that for a serological test in H. A. D. to be meaningful, one would expect it to measure the concentration of reagins presumed to be responsible for immediate hypersensitivity (or of the transfer factor(s) in delayed hypersensitivity), which are apparently the main factors related to the severity of the disease [1]. It would be also desirable to be able to measure independently the concentration of the blocking antibodies produced during hyposensitization treatment, since the ability of allergic patients to tolerate progressively increasing doses of the allergen after this therapy has been attributed to the protective capacity of these antibodies [2]. It ought to be pointed out, however, that from the experimental data accumulated within recent years [3], it has become evident that blocking antibodies are produced not only during treatment but also "spontaneously" by non-treated allergic individuals and that the reason for the failure in the past to recognize their presence in such sera was due to their very low concentration in these sera and to the insensitivity of the in vivo tests used for their demonstration.

General Properties of Antibodies.

As a general background for this discussion, it is proposed to give at this juncture a brief summary of the characteristic features of con-

ventional antibodies of the precipitating type. In the past, antibodies were thought to belong either to the electrophoretically slow migrating γ_2-globulins or to the faster γ_1-globulins. Since the introduction of immunoelectrophoresis [4], it has been demonstrated that antibodies produced even to a simple hapten represent an electrophoretically heterogeneous population of globulin molecules extending from the region of the slowest migrating γ-globulins into the region of β-globulins [5]. From a combination of the results of immunological, ultracentrifugal and immunoelectrophoretic studies, it has been generally accepted [6] that immunoglobulins comprise three major groups of proteins designated as 7S γ-globulins, 19S γ-globulins (γ_{1M} or β_{2M}), and γ_{1A}- (or β_{2A}-) globulins. The 7S and 19S γ-globulins have molecular weights of the order of 160,000 and 1 million, respectively. The γ_{1A}-globulins consist mainly of 7S components but contain also faster sedimenting moieties with sedimentation coefficients of the order of 10, 13, and 15S. These latter components as well as the 19S globulins are dissociated into 7S units on treatment with mercaptans (0.1M), under conditions where the 7S globulins do not undergo any detectable change. Each of these three groups of immunoglobulins consists of antigenically distinguishable subgroups corresponding to the two major types of Bence-Jones proteins, and molecules bearing one of the two type specific determinant groups are present in the three classes of γ-globulins. Besides these type specific determinants, each of the three immunoglobulins has both common determinants and specific antigenic groups which are absent in the others, and thus specific antisera for each sub-group of proteins can be prepared [36].

Skin-sensitizing and Blocking Antibodies.

Most of the commonly accepted biological and physicochemical properties of skin-sensitizing antibodies in sera of individuals sensitive to grass- and ragweed pollens can be summarized with the aid of the data given in Table I. In addition, it appears that blocking antibodies, and to a lesser extent, possibly also, skin-sensitizing antibodies may participate in the passive hemagglutination reaction. Moreover, the precipitins detected on concentration of serum fractions rich in blocking antibodies [7] have been identified with the latter [3].

The skin-sensitizing as well as blocking antibodies are defined in terms of their specific in vivo reactions with the allergen(s). Clearly, therefore, for any in vitro test used for the detection and quantitation of either of these two types of antibodies to be reliable, it is necessary to demonstrate that the observed serological manifestation is due exclusively to the factor which is also responsible for the in vivo reaction. This problem is rendered particularly difficult because of the complex antigenic composition of all allergenic preparations used to date in these tests and because of the likelihood that during hyposensitization treatment (and possibly also prior to such treatment) additional antibodies might be produced by the allergic individual against antigenic

Properties of Antibodies in Allergic Sera

TABLE I

Property	Skin-Sensitizing Antibodies	Blocking Antibodies
Fixation to human skin	+	
Passage through placenta	—	+
Thermal stability	poor	good
In vitro combination with allergen	+	+
Measured by passive hemagglutination	?	+
Electrophoretic mobility	γ_1	γ_2
Sedimentation coefficient	larger than 7S	7S
Absorbed with specific immune serum to	β_{2A}-globulins	γ-globulins
Inactivation with mercaptans (0,1 M).	+	
Degradation with papain	slow	fast

groups not involved in the direct skin reactions or in the blocking of the Prausnitz-Küstner test.

In Figure I are given a number of hypothetical models which may represent the different types of antibodies formed by an allergic individual. In principle, both reaginic and blocking antibodies may be either univalent or divalent, as regards the number of sites capable of combining with the allergen (denoted here as A. C. S.). However, by necessity the reaginic antibody must possess at least an additional site with the unique property of becoming fixed to tissue (denoted here as S. S. S.).

On the basis of the lattice theory [8, 9], the reactions between polyvalent antigen and divalent antibody molecules would be expected to result in the formation of multimolecular antibody-antigen aggregates provided the bonds involved in this combination are stable. Moreover, if the combining masses are in sufficient amount, these aggregates would be expected to precipitate and to be readily detectable by the naked eye, or by more sensitive methods such as light scattering. On the other hand, if these antibodies are univalent, only small soluble complexes consisting of one antigen molecule and a number of antibody molecules corresponding to the valency of the antigen would be formed. Similarly, one could not expect to detect large insoluble antigen-

antibody complexes, irrespective of the valency of the antibody, if the antigen were univalent, such as in the case of a hapten. Nevertheless, the latter difficulty could be generally overcome by coupling a number of univalent antigens or hapten molecules with a polyfunctional reagent (such as polylysine or a carrier protein) and creating thus a polyvalent antigen.

The reactions between conventional antibody and antigen molecules have been shown to be reversible and to be represented by a relation of the type

$$Ab + Ag \rightleftarrows (Ab)_x(Ag)_y$$

where Ab and Ag represent free antibody and free antigen molecules and $(Ab)_x(Ag)_y$ represents antibody-antigen complexes composed of x and y molecules of bound antibody and antigen, respectively. It is worth pointing out again that irrespective of the valencies of the antigen and antibody, no *in vitro* test could be devised unless the bonds formed on combination between these antigen molecules are stable, i. e. unless the binding constat, K, between antibody and antigen molecules is sufficiently large so that the association reaction is predominant. Obviously, one may also visualize the possibility that allergic antibodies could be divalent and might have a high affinity for the allergen, but that they may be present in exceedingly low concentrations; if this were the case, the amount of aggregates formed would be too small for detection by the standard precipitin tests.

Although all these concepts are accepted these days, as late as 9 years ago there was no clear cut evidence that combination between reagins and allergens did actually occur in vitro, though the available experimental data suggested that combination occurred between blocking antibodies and allergen [10]. This question has since been solved with the help of specific immunosorbents synthesized by coupling grass and ragweed allergens via covalent bond to polystyrene [11, 12], red cells [12, 13], and cellulose [14, 15]. Absorption of allergic sera with these immunosorbents was shown to result in the complete removal of skinsensitizing and blocking antibodies, demonstrating thus that firm combination occurred also in vitro between reagins and allergens. Moreover, as with conventional antibodies, it was shown that this reaction was reversible, since it was possible to displace the reagins off the immunosorbent with serum fractions rich in blocking antibodies [16]. Although these results were encouraging as regards the possibility of developing an in vitro test for the detection of reagins, they indicated clearly that the serum components responsible for reaginic activity were present in exceedingly low concentration, which was estimated to be well below 0.1 mg/ml. Consequently, it was obvious that the amount of material involved was too small to be detected by a direct serological technique, such as the precipitin test or the Ouchterlony method, and that one would have to resort to an indirect in vitro procedure for the possible demonstration of reagins by exploiting either (i) their proporty of behaving as an antibody in relation to the allergen, or (ii) their ability

to become fixed to cells and to lead to cell injury on further combination with the antigen.

No attempt will be made here to give a complete and historical review of all the procedures used for the development of an in vitro test for the determination of antibodies in allergic sera; such a survey can be found elsewhere [17]. Suffice it to say that the passive hemagglutination procedures have assumed the most prominent role amongst all these methods. The mechanism of the hemagglutination reaction was recently studied in detail [18] and it was demonstrated that the antibodies responsible for this reaction are divalent, i. e. of the types shown in Figs. 1(b) or 1(d), which is in conformity with the framework theory. Moreover, univalent antibody fragments, prepared by chemical and or enzymatic degradation of divalent antibodies, were shown to lead to the inhibition of the hemagglutination reaction, an effect which can be also obtained with free, soluble univalent or polyvalent antigens. However, in view of the recognized multiplicity of antibodies produced in the allergic response, one must be cautious in attributing this reaction to the blocking and/or skin-sensitizing antibodies. As has been stated, neither of these antibody factors has as yet been isolated in a pure state and, therefore, there is no direct evidence that the hemagglutination reaction measures the concentration of either skin-sensitizing or blocking antibodies. Nevertheless, from an analysis of the available evidence obtained with serum fractions, it can be inferred that the major portion of the hemaggglutinins in an allergic serum represents antibodies of the blocking type and that only a minor portion of the hemagglutinins might be identifiable with reagins, which would then be represented by molecules of the type shown in Fig.1(b). Some workers have repeatedly challenged this suggestion and have stated that reagins do not parti

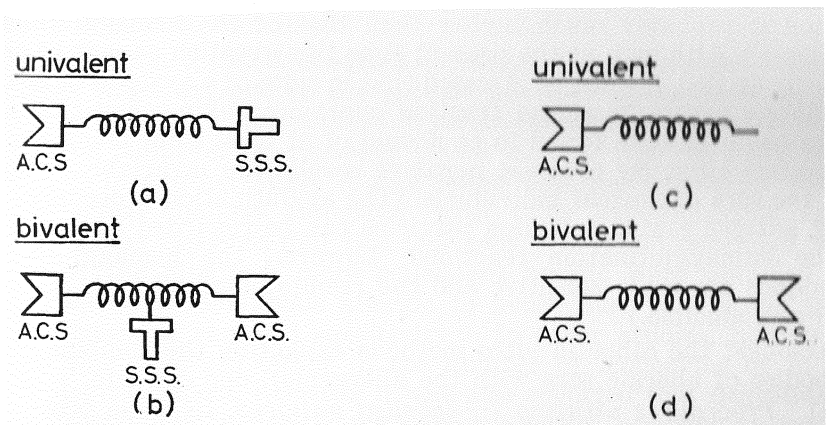

Figure 1.—Schematic representation of different hypothetical models of reaginic and blocking antibodies. A. C. S. and S. S. S. refer to antibody combining site and skin sensitizing site, respectively.

cipate in the hemagglutination reaction. If this were indeed the case, one would have to conclude that reagins are univalent antibody molecules of the type represented in Fig. 1(a). The main argument offered in support of the latter concept is based on the fact that in general the hemagglutination titer of serum fractions rich in reagins is very low by comparison with the skin-sensitizing titer. It ought to be stressed, however, that the disparity between these two titers does not constitute by itself definite proof that the two activities are attributes of different molecules, since the sensitivity of the Prausnitz-Küstner method is probably much higher than that of the hemagglutination technique [3]. Although at the present time this question cannot be satisfactorily settled, it must be stressed that in spite of a systematic search, no allergic serum or serum fraction containing skin-sensitizing antibodies has ever been found to be devoid of at least some small hemagglutination titer. By contrast negative results are invariably obtained with the sera of normal individuals. (The agglutination procedures utilizing antigen-coated collodion particles can be regarded as variations of the hamagglutination reactions; however, the hemagglutination techniques are more sensitive and the results obtained with them are more reproducible.)

Amongst the other in vitro methods exploiting the combination of antibodies of allergic sera with the allergen(s), one can cite.

(i) *Procedures utilizing I^{131}-labelled antigens.* In these procedures the soluble complexes formed between antibodies and the radio-isotope labelled antigen are precipitated either with a potent antiserum to human immunoglobulins [19] or with ammonium sulphate at 40 % saturation [20]. Radioimmunoelectrophoresis, as used by Dr. ARBESMAN and his colleagues [21], may be considered to be a refinement and an extension of these methods; it clarly demonstrates the multiplicity of the antibodies detected by this highly sensitive technique. However, as in the case of the hemagglutination reaction, these procedures do not demonstrate unambiguously the nature of the antibodies involved, though in all probability the bulk of the antibodies detected are of the blocking type.

(ii) *Complement fixation.* The in vitro combination of antibodies of allergic sera with the corresponding allergens has been demonstrated by complement fixation studies [22]. However, no simple relation was found between the amount of complement fixed and the amount or type of antibody present in these sera; nevertheless, in general the titers were higher with sera from treated allergic individuals.

(iii) *The use of cellular elements of the blood.* In addition to these classical immunological techniques, a number of methods based on reactions involving the cellular elements of the blood have been explored in an attempt to develop an in vitro method for the detection of skin-sensitizing antibodies. Thus, incubation of the blood of allergic individuals with the homologous allergen resulted in the release of histamine from leucocytes [23], [24]. This reaction has been shown to be specific; it could be produced only with the blood of allergic individuals and only

with the allergen to which they were sensitive. Moreover, maximal histamine release occurred at an optimal concentration of allergen which seemed to be related to the skin-sensitivity of the allergic individuals. A similar reaction was also obtained with the leucocytes of normal individuals (or rabbits) provided that the leucocytes were incubated with the allergic serum prior to the addition of the allergen [25]. These findings would suggest that skin-sensitizing antibodies have also affinity for leucocytes, and this would explain the success of the transfer of hypersensitive reactions of the immediate-type with viable leucocytes or with their extracts [26].

It was claimed that leucocytes of allergic individuals show peculiar reactions when exposed to the specific allergen. Thus, the number of leucocytes was found to decrease in the blood of ragweed sensitive individuals on addition of ragweed pollen extract due to lysis of the cells [27], [28]. In another study it was found that the leucocytes undergo morphological changes associated with their immobilization and or lysis [29]. It was reported that this leucocytolytic effect depended on the nature of the allergen: more consistent results were obtained with leucocytes from individuals allergic to house dust or timothy pollen, than in cases of ragweed sensitivity [27]. In other studies, however, no change was observed either in the number of leucocytes or in their morphological aspect. Recently it was also reported that degranulation of rabbit basophils, incubated with allergic serum, occurred on exposure to the specific allergen [30]. These results, however, have not been consistently reproduced [31]. Due to these contradictory results and to the lack of reproducibility, at the present time none of these seems to be satisfactory and suitable for the unequivocal demonstration of allergic reactions. The same consideration holds for another observation, namely for the agglutination of thrombocytes suspended in allergic serum which was claimed to occur on addition of the specific allergen [32].

In spite of the rather low reproducibility of these procedures, it would appear that the reactions involving the cellular elements of the blood are worth further investigation with purified serum fractions and might actually lead to a specific in vitro test for the detection of reagins. In this connection, Dr. SORKIN's observations on the cytophilic antibodies might be both relevant and most promising.

(iv) *Fluorescein-labelling techniques.* Combination of antibodies produced by allergic individuals with ragweed pollen constituents was recently demonstrated in two studies [33], [34] with the help of the fluorescein-labelling technique. In one study globulin preparations of allergic sera (isolated by precipitation with 20 percent ethanol) were conjugated with fluorescent dyes, and were shown to react with ragweed antigens in skin biopsies of allergic individuals [33]. In another study antibodies to human γ-globulins were labelled with fluorescein and were shown to stain pollen grains which had been previously exposed to allergic sera. Since, as has been shown recently [7], [35], sera of non-treated allergic individuals always contain blocking antibodies in addition to reagins, it

cannot be concluded whether these reactions were given by reagins, by blocking antibodies, or by both.

SUMMARY

The nature of antibodies in sera of allergic individuals and the methods used for their in vitro detection have been briefly summarized. References to detailed surveys of the literature have been given.

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(i) Recently a double-layer agglutination procedure, has been developed [HUGES, A. P., and AUGUSTIN, R.: *Nature*, 202: 668 (1964)] for the detection of antibodies in allergic sera which shows great promise of being capable of measuring reagins *in vitro*. In this method, human leucocytes are first incubated with the testserum, then treated with excess antigen and finally exposed to an antiserum containing precipitating antibodies to the antigen. Agglutination of leucocytes results if the test serum contains antibodies against the homologous antigen. Positive results were obtained with sera of non-treated hay fever patients, as well as with precipitating antibodies to pollens which were devoid of reaginas

(ii) According to a new nomenclature [*Bull. Org. mond. Sante*, 30: 447 (1964)] the three classes of human immunoglobulins, referred to on p. 166, should have been referred to as γG, γA and γM (or IgG, IgA and IgM) instead of 7S γ, γ-and 19S-globulins, respectively.

APPROACHES TO THE PROBLEM OF DETECTING REAGINS IN VITRO

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Three classes of globulins in serum have been found to be associated with antibody activity. They were characterized by immuno-electrophoresis and sedimentation constants and designated as γs (or γ), γ_1A (or β_2A) and γ_1M (or β_2M) globulins. In atopic conditions such as asthma, hay fever and urticaria, skin sensitizing serum antibodies known as reagins are often detectable. Recent work by several groups [1-10] has demonstrated that reaginic antibodies belong to the class of γ_1A immuno-globulins. Although the most conspicuous property of the reagins is their ability to become fixed to epithelial and other human and primate tissue cells, very little is known of the nature of the cell-fixing site. Many biological investigations have shown the significance of reagins in anaphylactic sensitization of skin in man. Yet their mechanism of action is still largely unknown.

STANWORTH [11] reviewed recently the various attempts that have been undertaken to replace the Prausnitz-Küstner method of detecting skin-sensitizing antibody in allergic sera by a suitable *in vitro* test. Lack of sensitivity of the methods may have been part of the reason for the failures. It is more likely, however, that several of the reported tests have in fact failed because they were based on the interaction of the allergen with a presumably common structure both in the reagins and the blocking antibodies, that of a similar or identical antibody combining site.

Amongst the several possible approaches to the measurement of reagins *in vitro,* the *indirect method* introduced by KATZ and COHEN [12] seems to be one of the most promising ones. It was discovered and confirmed by other authors that blood leucocytes of allergic patients released histamine on addition of the allergen. Refinement in histamine assays has also reduced the blood volume required for assay, thereby providing this method with an additional advantage. Since the blood cells can be washed several times before testing for histamine release

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it may be assumed that cell-fixed reagins, after interacting with the allergens, are responsible for triggering off the histamine release. Recently VAN ARSDEL and SELLS [13] succeeded in passively transferring into normal human blood leucocytes *in vitro* with allergic sera the capacity of subsequently producing histamine release upon interaction with allergen.

Our own attempts to measure reagins *in vitro* are essentially based on the discovery of cytophilic antibody in sera of immunized animals [11, 15, 16, 17]. The term "cytophilic antibody" has been proposed by us to describe a γ-globulin component of serum, which becomes attached *in vitro* to certain cells in such a way that these are subsequently capable of specifically adsorbing antigen. Reagins may be regarded in this sense as cytophilic antibodies, although no positive correlation between cytophilic activity and anaphylactic sensitization of cells has been demonstrated in rabbits and guinea pigs ([16] and SORKIN, unpublished results).

It was thought that primary interaction of cell-fixing and cell fixed reagins could perhaps be measured directly by 1) measuring cell bound reagin on blood leucocyte through interaction with isotope labelled allergen or/and by 2) coating first tissue cells, such as normal blood leucocytes, human epithelial cells (e. g. Hela cells) or human dander with reaginic antibody and then let these cells interact with isotope labelled allergen (cytophilic antibody technique). Already SAMSOE-JENSEN and HAUGE-KRISTENSEN [18] and AUGUSTIN [19] have indicated that blood leucocytes or tissue cells can absorb out skin-sensitizing antibody from sera. It is surprising that no serious attempts to assay reagins have been made so far using this distinct quality of reagins, namely to fix onto cells and to subsequently react with the allergen.

So far only few results with human leucocytes and sera are available. Model systems in rabbits, as shown below, although not necessarily dealing with reagins, clearly indicate, however, some prospects for a direct *in vitro* measurement of reagins.

Blood leucocytes of rabbits immunized with human serum were isolated by the Heparin-Dextran method. 25 ml. blood were immediately mixed with 5000 units of heparin/0.5 ml. saline and 2.5 ml. Dextran solution (Macrodex, Pharmacia, Uppsala, Sweden). After standing for one hour at room temperature at an angle of 45 degrees, the supernatant fluid with the leucocytes was withdrawn and centrifuged at 400 g for 7 minutes. Further lots of leucocytes were obtained by 2 more centrifugations of the remaining blood. The combined leucocytes were removed after washing with 10 ml. of a solution containing 50 ml. 5% normal rabbit serum in Gey's solution and 2.5 ml. of 20,000 units of heparin.

For evaluating directly the level of cell-bound antibody about 30-50 10^6 leucocytes, which still contained sometimes up to 50 10^6 erythrocytes were:

a) directly incubated with 0.5 microgram isotope labelled I^{131} human serum albumin (I^{131}-HSA) at 37° C. for one hour and then washed 4 times with Gey's solution,

b) first treated with methanol for 5 minutes at room temperature, washed three times with Gey's solution and then incubated with I^{131}-antigen, as under a).

Blood leucocytes and red cells from normal rabbits served as controls. The results are illustrated in Table I.

TABLE I

Cell-bound antibody in rabbit blood

Cells	Animal No.	Antigen (I^{131}-HSA) adsorbed onto cells: No methanol		Antigen (I^{131}-HSA) adsorbed onto cells: Methanol pretreatment	
			cts/min.		cts/min.
Blood leucocytes from immunized rabbits ($30\text{-}50 \times 10^6$)	66	a)	164	a)	1441
	78	a)	677	a)	903
	85	b)	507	b)	353
	89		37		1492
	02	c)	2027	c)	2863
	06		5263		3688
Erythrocytes from immunized rabbits (5×10^8)	66	a)	60	a)	691
	78	a)	15	a)	87
	85	b)	31	b)	12
	89		8		16
	02	c)	0	c)	7
	06		0		10
Normal blood leucocytes ($30\text{-}50 \times 10^6$)		a-c)	33	a-c)	42
Normal erythrocytes (5×10^8)		a-c)	1	a-c)	3

Applied antigen in experiment a) 0.5 microgram I^{131}-HSA = 2,751 cts/min.
Applied antigen in experiment b) 0.5 microgram I^{131}-HSA = 4,643 cts/min.
Applied antigen in experiment c) 0.5 microgram I^{131}-HSA = 11,103 cts/min.

The blood leucocytes of these immunized and boosted rabbits carried significant amounts of cell-bound antibody. It is significant that fixation of antigen is not due to red cells present in the leucocyte preparations since in nearly all of the rabbits even 5×10^8 red cells had no appreciable antigen binding capacity. The effect of methanol was somewhat variable. In four out of six blood leucocyte preparations of immunized animals methanol increased the fixation of antigen onto cells, while no significant change occurred with normal cells. These results demonstrate definitely that the level of blood leucocyte antibody can be measured directly by interaction with isotope labelled antigen.

Our findings [16] of the existence of cytophilic antibodies in antisera to various antigens suggested that part of the antigen fixing capacity of blood leucocytes from rabbits may have been caused by fixation of cytophilic antibody on to the blood leucocytes. The sera of immunized rabbits were therefore tested in our "Standard Technique" for the titration of cytophilic antibody but using normal blood leucocytes and for control purposes spleen cells from normal rabbits [14, 15]. Table II gives the results.

TABLE II

Cytophilic antibody levels in the immunized rabbits

Type of rabbit cells used for antibody fixation	Sera Animal No.	Antigen (I^{131}-HSA) adsorbed onto cells cts/min.
Normal blood leucocytes (40×10^6)	66	69
	78	17
	85	7
	89	19
	02	27
	06	225
	Controls	7 - 12
Normal erythrocytes (5×10^8)	All immune and normal sera	7 - 22
Normal spleen cells (150×10^6)	66	637
	78	106
	85	21
	89	55
	02	305
	06	1705
	Controls	12 - 25

0,5 microgram I^{131}-HSA = 7,123 cts/min.

Cytophilic antibody levels in the immunized animals, when tested with normal nucleated spleen cells, showed the anticipated values. In contrast only relatively low cytophilic antibody titers could be measured with about 40×10^6 normal blood leucocytes. As was shown in Table I, a similar cell number from immunized rabbits had a much higher affinity for the antigen. These results suggest that the antigen binding capacity of blood leucocytes from immunized rabbits was due probably only to a relatively small extent to passive adsorption of circulating antibody onto such cells. The main contribution to antigen fixation in this instance must therefore have been caused by antibody producing cells in blood, as demonstrated earlier [20].

The described results show, nevertheless, that rabbit cytophilic antibody and, as discussed above, probably to a considerably higher extent the human reagins can become attached to blood leucocyte cells and be measured after interaction with isotope labelled antigen.

Further extension of the cytophilic antibody reaction has already shown that normal blood leucocytes can be replaced by human epithelial cells grown in tissue culture cells such as Hela cells or HEp 2 cells.

Rabbit anti-grass pollen sera (Timothy), obtained from Dr. R. AUGUSTIN, showed some cytophilic antibody activity. Also rabbit anti-penicillin sera (3 mg antibody/ml.), obtained from Dr. A. DE WECK, contained a small amount of cytophilic antibody when suspended human dander cells were used as adsorbing particles and a penicillin-I^{131}-HSA conjugate as antigen.

TABLE III

Adsorption of rabbit anti-penicillin and anti-HSA antibodies onto human dander

Serum	Penicilloyl-I^{131}-HSA adsorbed	I^{131}-HSA adsorbed
	cts/min.	cts/min.
Normal rabbit serum	14	12
Rabbit antipenicilloyl serum	126	17
Cytophilic rabbit anti-HSA serum	5245	3700

0.87 microgram I^{131}-HSA = 10,500 cts/min.
0.87 microgram Penicilloyl-I^{131}-HSA = 15,400cts/min.

Preliminary experiments with human blood leucocytes from a few patients allergic to Timothy-grass pollen and isotope labelled Timothy allergen showed some indications for increased adsorption of allergen as compared with normal cells (SORKIN and AUGUSTIN-unpublished). Blood leucocytes of 20 house dust allergic patients and 10 normal controls showed no significant differences when tested with the isotope labelled house dust allergen, prepared by Dr. L. BERRENS.

CONCLUSION.

A method for detecting cell-fixed and cell-fixing antibody in rabbit blood has been described. It is capable of measuring as little as 0.001–0.01 microgram of antibody Nitrogen in the cytophilic antibody test. After refinement and suitable modifications this method may eventually become applicable for measuring the primary interaction of blood leucocyte bound and circulating reagins *in vitro*.

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SYMPOSIUM

ASMA BRONQUIAL

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INTRODUCCION:

FACTORES ETIOPATOGENICOS DEL ASMA BRONQUIAL

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Fundación Jiménez Díaz. Instituto de Investigaciones Clínicas y Médicas. Madrid

La consideración del asma como una "enfermedad de la persona", que es la intención de este Symposium, supone una amplia serie de cuestiones cuando queremos hacer un análisis en el que se aproximen nuestros conceptos a la realidad polimorfa y con frecuencia oscura que nos brinda la clínica. Tales cuestiones son de diversa índole, y solamente a través de su examen sucesivo podemos llegar a formar un concepto amplio pero definido de lo que es la enfermedad.

En el sentido etiológico será interesante conocer la frecuencia de factores según la experiencia de diversos autores y países. En algunas formas etiológicas, por ejemplo en las polinosis, habrá diferencias de unos países a otros por el tipo de flora anemófila y las condiciones fenológicas, pero para una misma localidad los resultados serán muy similares porque se trata del tipo más seguro de desencadenante que aparece tanto en la anamnesis como en los tests cutáneos y en la transferencia pasiva. Las diferencias serán en cambio muy marcadas, según el observador, en sitios similares para otras sensibilizaciones, revelándose en ello las diferencias de criterio individual, que demostrarán con qué frecuencia las conclusiones son inseguras, y cómo muchas veces una influencia dudosa o de importancia relativa, dentro de los factores desencadenantes, es tomada como causa principal y constituye un "Wunschdiagnose"; la influencia de los hongos, de los alimentos y en menor proporción también la del polvo, es clara y segura muchas veces, pero insegura y aceptada con ligereza muchas otras. Con todo hay una proporción de casos que está en nuestra experiencia y en la de muchos autores alrededor del 25-30 por 100, en que siendo un asma no sintomático, sino primario, con similares características, no se puede demostrar con seguridad una influencia exo-alergénica. Hasta qué punto son asmas "infecciosos o bacterianos", o "asma intrínseco" el resto de los casos, es muy difícil de definir, como también lo es principalmente el saber lo que quiere decirse con estos términos convencionales. Otros factores al lado o fuera de la sensibilización, como la infección, la constitución, el equilibrio endocrino-vegetativo, los factores emocionales y meteorológicos, tienen que ser también considerados; pero ¿qué significa que

circunstancias tan distintas provoquen una reacción asmática estereotipada constituyendo en su conjunto lo que llamamos un "modelo de reacción"? En el asma, como en otros paroxismos funcionales, una serie de fenómenos fisiopatológicos aparecen asociados independientemente de la cualidad del factor desencadenante; en esquema, la reacción está integrada por la tétrada de secreción, edema, vasomoción y miocontracción. En la *patogenia* de la crisis asmática estos fenómenos intervienen en proporciones variables, y pensamos que son el producto de la liberación de mediadores químicos y de modificaciones en ciertos sistemas enzimáticos. Pero parece posible que esta descarga pueda hacerse en el asma, como pasa en la urticaria por ejemplo, por mecanismos diferentes entre los cuales está el choque inmunológico. Es necesario aclarar cuáles entre esos mediadores tiene la mayor importancia, cosa no aclarada tampoco en el caso del shock anafiláctico experimental; como lo es también conocer los factores que inhiben y los que facilitan esa respuesta de descarga.

En el sentido de la *fisiopatología* del asma, los estudios de dinámica respiratoria han supuesto un gran progreso en el diagnóstico, en el pronóstico, y para fundamentar una terapéutica. No obstante, es necesario conocer la repercusión, en el organismo total, del asma a través de la insuficiencia respiratoria, no solamente en el aporte de oxígeno, aireación del carbónico, y, por consiguiente, del equilibrio ácido-base e iones, sino también sobre las funciones metabólicas y principalmente sobre las funciones de los centros nerviosos. Cuando se atiende al *pronóstico* del asma es cuando se ve más claro que, generalmente, incluímos en él cosas diferentes. Ultimamente ha habido varios autores que se han alarmado por la frecuencia creciente de casos de muerte en los asmáticos que antes era considerada como excepcional. Nuestro análisis de los casos observados nos permite concluir que la muerte por asma primario sigue siendo excepcional (en el 0,08 % de nuestros casos), y que los casos de muerte que no es propiamente asmática llegan a ser el 1,3 %, cerca de 20 veces mayor. Pero en esos casos la evolución no fue simplemente disneica, sino que se acompañaba de episodios repetidos de cianosis, y edemas, con insuficiencia respiratoria global y acidosis. Son casos que si en una época primera tuvieron un carácter de asma funcional, más adelante tuvieron caracteres de orgánicos (bronquitis, fibrosis), seguramente con alteraciones de membrana, originando el bloqueo alveolocapilar. ¿Se puede considerar que esto era asma en evolución orgánica, o más bien un proceso orgánico (bacteriano, viral) que comenzó con síntomas funcionales y se desarrolló más adelante? En todo caso estos tipos de asma parecen diferentes de los otros. El *diagnóstico* diferencial del asma con otras enfermedades respiratorias en las que hay manifestaciones asmoides, muy fácil en muchos casos, es en otros muy difícil; hay una zona en la que la decisión entre asma bacteriano, o intrínseco, bronquitis crónica, fibrosis, y otras enfermedades orgánicas broncopulmonares es muy difícil y depende en gran parte del pensamiento subjetivo del observador. El criterio que parece más justo es la observación de la reversibilidad farmacológica del cuadro clínico y de las alteraciones en la función respiratoria; pero

En la mayor parte de estos casos el médico necesita más bien que una definición global, analizar y conocer hasta qué punto los diversos factores intervienen, es decir, la proporción de lo orgánico y lo funcional, la existencia de factores alergénicos, la infección, el grado de obstrucción bronquial, las peculiaridades de la secreción mucosa y la repercusión espirométrica, y sobre la ventilación de la sangre, la función circulatoria, el equilibrio ácido-base, etc. En el momento actual el médico que trata a asmáticos necesita manejar estas técnicas y no puede limitar su papel simplemente a la exploración de las sensibilizaciones. Así, la *terapéutica* que debe derivar de este análisis puede aportar sus mayores rendimientos; el tratamiento desensibilizante ha adquirido en los últimos años nuevas técnicas; subrayemos el interés de la terapéutica repositoria, los antibióticos y los esteroides cortisónicos, la supresión de alergenos en la atmósfera del enfermo, la gimnasia respiratoria, el tratamiento psicológico, los aparatos de respiración controlada, el tratamiento de la acidosis, etc.

Es indudable que en gran parte nuestros avances en el aspecto teórico tienen una gran importancia y son una esperanza del futuro, pero la práctica clínica en el asma exige una revisión de estas y otras cuestiones que van a ser tratadas a continuación. Si es cierto que a medida que conocemos mejor las cosas aprendemos a actuar mejor, es igualmente verdad la recíproca, actuando aprendemos, y la práctica terapéutica actual más compleja viene enseñándonos muchos aspectos de la enfermedad asmática.

THE NATURAL HISTORY OF ASTHMA

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The main factors predisposing to and precipitating asthma are heredity, exposure to allergens, naso-respiratory infections and stress (SCHWARTZ 1952, WILLIAMS 1959, SHERMAN 1963).

Figure 1 shows the age of onset of asthma in a group of unselected asthmatics found in a survey of the prevalence of asthma (WILLIAMS and HIGGINS, 1958). It shows that asthma commonly commences in childhood or young adult life and that before puberty boys develop asthma more frequently than girls as is generally accepted (RACKEMANN and EDWARDS 1952, SMITH 1961, BRODER et al 1962). After puberty and until after the menopause women develop asthma more frequently than men (VAUGHAN and BLACK 1954, WILLIAMS 1959). The reasons for the sex differences are not known.

The course of the natural history of asthma has been most excellently studied by Dr. FRANK RACKEMANN who has shown that the asthma of childhood tends to improve often clearing completely in young adult life, but then tends to return in later years (RACKEMANN 1958). RACKEMANN and EDWARDS (1952) followed up 449 children after about 20 years and found that 52 % became free from asthma, another 19 % could successfully avoid the cause; thus nearly three-quarters (71 %) lost their asthma. A quarter still had asthma but this was serious in only 11 %. Four had died of asthma, a mortality of less than 1.0 %. The average age at which freedom from symptoms occurred was 15 years but varied between 5 and 30 years; there was no difference in the sexes. Winge Flensborg of Copenhagen, in 1945 found that of 131 children with asthma followed up to the age of 18 years, 41 % had lost their asthma. Grant of Cardiff in 1957 found that of 152 young men and women with a history of asthma attending the University of Wales, 42 % had by then become free of attacks. Recently RYSSING and WINGE FLENSBORG (1963) on following-up 442 asthmatic children at ages 15-25 years found that 37 % had become symptom-free for 12 months or longer, 9 % had cough and/or occasional dyspnoea of a non-asthmatic character while 54 % still had asthmatic symptoms either intermittently or continuously. The severity of the asthma affected the outlook; in those who had had mild asthma 50 % became free; of those with severe

asthma only 28 % became free. Of those with negative skin tests (35 % of the total) 47 % became free as against 40 % with one manifest allergy, 33 % with two and 19 % with three manifest allergies. No patient with four or five manifest allergies became symptom-free. Only 1 out of 30 children with complicating pollen allergy and only 1 out of 12 with mould allergy became symptom-free.

In *adult* asthmatics of the intrinsic type followed-up for at least 15 years, RACKEMANN (1958) showed that of 273, 22 % had cleared, 44 %

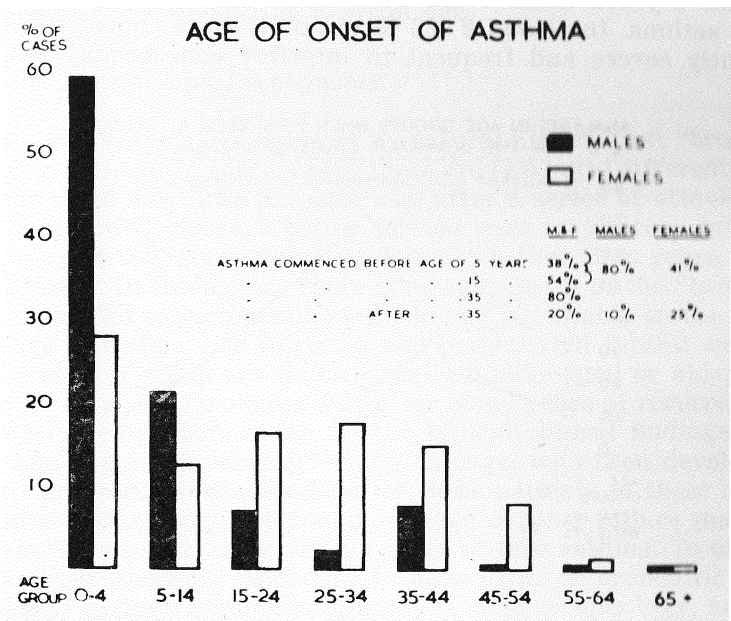

Fig. 1

were improved, 35.4 % were unimproved and 8.5 % had in all probability died of their asthma. The prognosis in general is obviously less satisfactory in adults than in children. Discussing his unimproved cases RACKEMANN wrote "there was found no sign to indicate the more serious prognosis in these cases". We must remember that the terms extrinsic and intrinsic indicate an aetiological classification and should not be used too rigidly to guide us in our prognosis. RACKEMANN (1958) pointed out that sinusitis and nasal polypi were more common in the unimproved than in the improved group; sinusitis occurred in 39 % and polypi in 27 % in the unimproved group, whereas in the improved group these were 12 % and 5 % respectively. This syndrome of asthma of the intrinsic type with sinusitis, nasal polypi and often aspirin sensitivity is well-known, and generally accepted as carrying a poor prognosis. WALTON et al (1951) followed 22 aspirin sensitive cases for 4 years during which 5 died, i. e. a mortality of 23 %. WALTON pointed out that in the 5 who died, their asthma had been intractable and they had been

subject to status asthmaticus, while in those with mild asthma there was no apparent change at the end of 4 years. It would appear that it was the severity of the asthma which really indicated the prognosis and not the aspirin sensitivity. The importance when assessing prognosis of grading asthma by its severity irrespective of the aetiology is well brought out by BRUCE PEARSON (1958). His 625 patients of all ages with active asthma he graded into 4 groups. In *Group 1* (5 % of total) were those *prevented from working* on account of their asthma and during the 6 years under observation 30 % died directly or indirectly of their asthma. In *Group 2* (23 % of total) were those with attacks sufficiently severe and frequent to interfere considerably with their

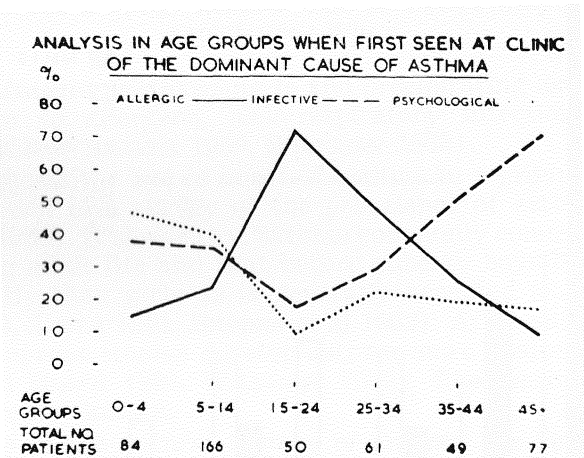

Fig. 2

life necessitating *long periods away from school or work,* 8.2 % died of asthma. In *Group 3* (57 % of total), were those *liable to occasional attacks of some severity but otherwise able to lead a normal life* with the aid of symptomatic remedies; 1.4 % died of asthma. In *Group 4,* (15 % of total) were those able to control attacks easily with symptomatic remedies and in this group there were no deaths. More recently BRUCE PEARSON (1963) has suggested a grading of asthmatics based on simple lung function tests.

BRUCE PEARSON (1958) found as have others (Ogilvie 1962) that the proportion of severely affected asthmatics rose with increasing age and with increasing age infection played a more important part both in the precipitation and maintenance of asthma.

Figure 2 sets out the frequency of the three main precipitating causes of asthma, the dominant precipitating causes as found at Cardiff in the different age groups (WILLIAMS et al 1958) and indicates, I believe, the natural history of asthma in relation to these factors.

It can be seen that extrinsic allergens, as the dominant precipitating causes, are not common in infants, are the commonest precipitating causes in young adults but become relatively infrequent causes again

in those over 45 years of age, i. e. infants tend to grow into their allergies (inhalant), then in later life tend gradually to outgrow them. Naso respiratory infection is a relatively common cause in infants and children, an uncommon cause in young adults but by far the commonest precipitating cause of asthma in those over 45 years of age, i. e. children outgrow their tendency to colds precipitating asthma, but later, in the middle and older age groups colds again commonly precipitate asthma. The psychological factor, a common precipitating cause in infancy is uncommon in young adults, and then becomes a relatively common precipitating cause in those over 35 years of age.

Bronchitis, Asthma and Emphysema.

It has been suggested as part of its natural history that "bronchitis" may lead to asthma. In this concept there are two quite different groups. In the one there are those patients who after a series of attacks of so called "bronchitis", perhaps better termed naso-respiratory infections, usually of relatively short duration, months rather than years, develop typical asthma. In these cases there is no suggestion of the "bronchitis" having given rise to permanent lung damage but that a series of naso-respiratory infections had initiated and perpetuated typical asthma.

In the second group are those (usually middle-aged or elderly men, and usually cigarette smokers) who after many years of recurrent bronchitis develop emphysema with all the typical clinical findings of permanent and usually considerable lung damage, who then develop their so-called "asthmatic" attacks. Most of these patients in these so-called "asthmatic" attacks will be found to have cardiac failure due to cor pulmonale. To me such cases are not cases of true asthma. To call these cases "asthma", asthma secondary to bronchitis, or bronchitic asthma or secondary asthma only results in a confusing terminology, as did the terms cardiac asthma and renal asthma years ago. Sometimes acute "asthmatic" attacks in these emhysematous patients are due to an acute respiratory infection, to an acute purulent bronchitis as a terminal event as I have seen, again no typical asthmatic findings either clinically or pathologically. One thing we can be sure about is that pathologists can recognize asthma when it occurs at or shortly before death.

It has also been widely held and over many years (Hyde Salter, 1860) that asthma eventually leads to emphysema. It is important to distinguish between over-distended lungs, distensive emphysema, and the type of emphysema where there is destruction of the lung tissue, destructive emphysema (FLETCHER, 1963). Distensive emphysema undoubtedly occurs in an asthmatic attack especially so in status asthmaticus, but there is no reason to doubt that it is only transitory. It is the problem of destructive emphysema in relation to long standing asthma which is difficult to be sure about clinically. Before attributing lung damage to long-standing asthma one must exclude other possible causes of lung damage, such as smoking, previous pneumonia or old pulmonary tuberculosis.

The evidence that cigarette smoking frequently plays a part in bronchitis and destructive emphysema is incontrovertible (R. C. P. London, 1962). I feel sure that heavy cigarette smoking has been responsible for the destructive emphysema of some of my male asthmatics (WILLIAMS, 1961) and that smoking can alter the natural history of asthma.

At my clinic 16 % of the male asthmatics smoked over 10 cigarettes a day, almost 5 % over 20 a day and these figures would have been higher if I had included men with undoubted bronchitis and emphysema who had had asthma previously. They were all heavy cigarrette smokers. In my female asthmatics the picture is quite different; only very exceptionally do they appear to go on to permanent lung damage and chronic cor pulmonale and only exceptionally are they heavy smokers. In a survey of 320 at my clinic, none smoked over 15 cigarettes a day, only 1 % over 10 a day while 94 % smoked less than 5 a day and 85 % had never smoked (WILLIAMS, 1961).

It was in 1949, 15 years ago, that Professor GOUGH introduced a new technique, the Gough-Wentworth technique of large lung section and asked me to let him have lungs of patients who in my view had died in status asthmaticus and had been typical asthmatics so that he could study whether or not they developed emphysema. The final proof of the end result of asthmatic illness on the lungs must of course be with the pathologists and later in the programme Professor GOUGH will give his findings.

Asthma as a Cause of Death.

After the pioneer paper of HUBERT and KOESSLER in 1922 it became established that asthma itself could cause death and asthma began to be established as a pathological entity.

Many have contributed to this subject since (THIEME and SHELDON, 1938; RACKEMANN, 1944; GAY, 1946; WALTON et al, 1961; WINER et al, 1950; WALZER and FROST, 1952; WILLIAMS, 1953; CARDELL, 1956; UNGAR, 1957; WILLIAMS and LEOPOLD, 1958; GOUGH 1961; FELDMAN, 1962; NEDER et al, 163; ALEXANDER, 1963).

The frequency with which patients die from their asthma is still a controversial subject and it may well differ in different climates (WILLIAMS, 1959). BULLEN of ROCHESTER, New York, in 1952 found that of 176 hospitalized patients who died and who had or had had asthma in the past that about one third died of their asthma. CARDELL and BRUCE PEARSON (1950) in reviewing their own cases and the literature considered that deaths from genuinely uncomplicated asthma would be less than 26 % of the total. MESSER and his colleagues (1960) from the Mayo Clinic found that of 304 asthmatic patients examined at necropsy, 11 % died of status asthmaticus, 10 % of some other asthmatic complication and 78 %, about three quarters, died of causes unrelated to asthma.

Recently 110 adults, all living in the City of Cardiff, 36 men and 74 women who attended my clinic between 1951-54 have been followed for 8 years or until death. During this time 21 died, 5 men and 16 women.

Of these 21 deaths, 8 (38 %) all women died in status asthmaticus, proven by autopsy in 5. The average age was 47 years and ranged from 32-66 years. Two deaths, both men, were due to pneumonia (both proven post mortem); the remaining 12 (57 %) died of illnesses unconnected with their asthma, i. e. carcinoma, coronary thrombosis, cerebral haemorrhage, etc. The original selection of these patients was that they should be undoubted cases of asthma and live within the Cardiff City boundaries. It is of interest that of the 74 women observed over 8 years, 8 or 11 % died of their asthma; of the 16 women who died during this 8 years 8, a half of them, died of their asthma.

132 Deaths from Asthma. Analised by Age & Sex

Age in years	Males No.	Females No.	Total No.
0- 9	1 (1)	3 (2)	4 (3)
10-19	—	1	1
20-29	1 (1)	5 (1)	6 (2)
30-39	7 (3)	17 (10)	24 (13)
40-49	8 (4)	17 (8)	25 (12)
50-59	16 (10)	18 (9)	34 (19)
60-69	10 (5)	22 (17)	32 (22)
70 +	2 (1)	4 (3)	6 (4)
Totals	45 (25)	87 (50)	132 (75)

Post mortems in 75 cases [figures bracketed ( )]; no post mortems in 57, but all confirmed clinically.

I have now (Table 1) collected 132 cases from the Cardiff district that have died in status asthmaticus with post mortem confirmation in 75. As has been found in some other British series (EARLE, 1953; HOUSTON et al, 1953; ROBERTSON et al, 1954; DUNNILL, 1960) female deaths from asthma preponderate over male deaths. Also, as in other series (ALEXANDER, 1958) there is a small group of deaths from asthma in infants, then very few deaths until after the age of 30 to 35 years when deaths in asthma become commoner. Ninety-two per cent of all these deaths occurred after 30 years of age and 73.5 % after 40 years of age.

In the large majority their final persistent distressing attack of status asthmaticus with its *persistent marked tachycardia* (WILLIAMS, 1963) in spite of all therapy made their death, with increasing experience, not unexpected. Occasionally, however, death occurred relatively suddenly and unexpectedly in an attack which did not appear as severe as previous ones. All had had previous attacks of status asthmaticus.

Thirty per cent had their onset of asthma before 20 years of age, in one third (33 %) their asthma commenced between 20 and 40 years of age, and, in another 30 % their asthma commenced after 40 years of age. The striking feature was not their age of onset but that almost all had a period of chronic incapacity varying from a few years to a few months with recurrent attacks of status asthmaticus before they died, i. e. they

had and intractable phase of their asthma, an incapacitating phase, one might call it an accelerated phase or even a malignant phase. In the remaining few, death occurred after only a very short illness but these had had previous attacks of status asthmaticus when they had been desperately ill. All these deaths from asthma were in cases of asthma of the "intrinsic" or infective type, and aspirin sensitivity was rare.

In retrospect in none of these cases could we say that death from

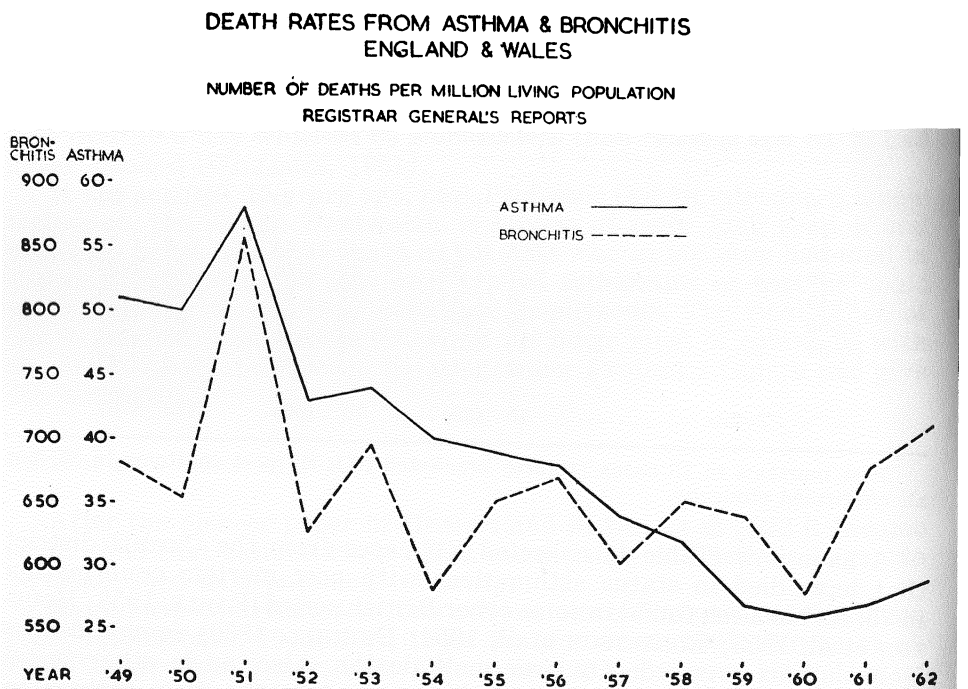

Fig. 3

asthma was entirely unexpected and certain generalities seem justified. The risk of death from status is higher in females than males, it is higher in infancy or after 30 years of age than in the period of young adult life and it is much higher in severe and incapacitated asthmatics than those less affected, very rarely occurring unless there has been previous attacks of status. However, much further work is required to enable us to predict more accurately the prognosis in asthmatics acutely ill and to understand more precisely the exact cause of death.

Figure 3 shows that the risk of death from asthma is not constant. The fall in the death rates from asthma correlates with the use of steroids. The death rates from bronchitis have not changed. I am sure you will agree with me that, in future, we will have to study the natural history of asthma as modified by the use of steroids.

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THE ROLE OF SENSITIZATION IN BRONCHIAL ASTHMA

GEORGE I. BLUMSTEIN, (*)

The title of this presentation makes it necessary to define certain terms as they will be used throughout this paper. Since our primary concern is solely with that type of asthma where sensitization is involved, the term asthma will be used synonymously with allergic, atopic, extrinsic or bronchial asthma, where the mechanism of sympton production is solely by way of antigen-antibody reaction. Inherent in this definition is a series of dynamic pathophysiologic tissue alterations that are responsible for the manifest symptoms and were or will be alluded to and elaborated on by other speakers. The patients with this type of asthma will be categorized as atopic [1] individuals.

The term sensitivity will be used synonymously with hypersensitivity and allergy to denote a heightened abnormal response to environmental allergens or antigens which result in bodily reactions. The provoking agent is innocuous to normal subjects and lacks the ability to produce visible responses in them.

Is the atopic asthmatic sensitized more easily than his normal nonatopic counterpart? BOTH BRUNNER [2] and COOKE [3] found it difficult to sensitize humans by the subcutaneous route with aqueous solutions of commonly encountered inhalants and pollen allergens. Yet when the proper antigen was, used, ascaris in Brunner's case [2] and guinea pig serum in Simon's instance [4], they were able to sensitize 60 and 100 % respectively of their subjects. BRUNNER felt that the nature of the antigen was more important than the atopic tendency as a determinant in sensitization. SIMON and RACKEMANN during their experiments demonstrated that the delayed or tuberculin-type sensitization invariably preceded the development of immediate type hypersensitivity. They too believed that no difference existed between the atopics and nonatopics in their capacity to become sensitized.

Yet the atopic individual seems to differ from his normal nonatopic counterpart in at least two important respects. The first is a qualitative one and pertains to the type of antitoxin produced in atopics in response to injections of diphtheria toxoid. The second is a quantitative one

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indicating a difference in incidence and time relationship in the development of immediate type hypersensitivity in atopics following stimulation with emulsified pollen extract. KUHNS and PAPPENHEIMER [6] showed that atopic individuals produced a nonprecipitating antitoxin following injections of diphtheria toxoid. This contrasts with the nonatopic who produced classical precipitating antitoxin. They also presented evidence to indicate that the nonprecipitating antibody was in all probability a reagin because of its ability to sensitize human skin, its heat lability and its persistence at passively transferred sites for several weeks. The second or quantitative factor relates to the ease of producing immediate-type skin reactivity in atopics. FEINBERG et al [6, 7, 8] demonstrated these variables by treating a group of atopics and nonatopics with emulsified pollen extracts for which no skin or clinical reactivity existed. They were able to produce immediate-type sensitivity in all 10 atopics, but only in 3 of 12 nonatopics. Furthermore, the atopics attained this sensitivity within the first few weeks following stimulation while it required a longer period for the non-atopics to demonstrate this type sensitivity. The reaction time for the development of delayed sensitivity was the same for both groups, usually 2 to 4 weeks. Many of the atopics developed only the immediate-type sensitivity. Furthermore, none of the clinically sensitive individuals developed delayed reactivity following specific emulsion pollen therapy. It was their feeling that delayed hypersensitivity was not a prerequisite for the development of immediate-type hypersensitivity.

Thus far we have considered sensitization to aqueous or emulsified extracts administered only by the parenteral route. Under ordinary circumstances sensitization in asthma does not occur in this manner but usually as the result of natural exposures to environmental and occupational allergens. Two representative circumstances are being selected to illustrate the manner in which sensitization evolves in patients suffering with asthma.

The first pertains to numerous recorded observation on groups of bakers who became sensitized as the result of exposure to flour during the course of their employment. Table I demonstrates the percentage of cutaneous and clinical reactions in the reported groups of bakers and the average duration of exposure in those having clinical sensitivity. A rather high proportion of persons working with flour become cutaneously sensitized, the percentage varying from 10 % in Hlavacek's series [13] to 54 % in Baagoe's [11] group but averaging out at about 40 %. On the other hand, clinical allergies develop in from 3 to 32.3 % or in about 50 % of those with skin sensitivity. COLMES et al [9] found a direct relationship between positive cutaneous reactions to flour and duration of exposure or employment. There also seemed to be an optimum average time within which clinical sensitivity usually develops [15], being shorter for coryza (8.4 years) and longer for asthma (13.2 years); the latter usually evolving from the former. Van DISHOECK and ROUX [14] also noted a positive correlation between the intensity of the exposure and the proportion of workers sensitized; it being larger (44 %) in the small

TABLE I

Duration of Exposure and Incidence of Sensitivity in Bakers

Author	Number of Patients	Cutaneous Reactions (%)	Clinical Reactors (%)	Average Exposure in Years
Colmes et al (9)	32	47	3	8.9
De Besche (10)			5 patients	15.8
Baagoe (11)	300	54	11	11
Salem and Dannfelt (12)	125	40.8	25.6	15
Hlavacek (13)	70	10	10	7-40
Dishoeck and Roux (14)	262	25	25.2	57 % in 10 yrs.
Schwartz (15)	35	100	100	10.8
Castberg and Mygind (16)	130	41.9	32.3	—
Linko (17)	328	17.7	20.1	11
Pestalozzi and Schnyder (18)	159	57	26.4	12.7

bakeries where the atmospheric contamination with flour was greatest and smaller (12 %) in the larger more modern, hygienic establishments. Opinions vary regarding what role, if any, the allergic predisposition plays in determining susceptibility to the development of cutaneous and clinical sensitivity. Those taking the affirmative side [11, 13, 16, 18] cite figures supporting their thesis. Others [12, 14, 15, 17] present equally convincing data indicating equal susceptibility towards sensitization in bakers without the family predisposition. Many of the cited studies were made in different eras, in different countries with divergent population groups, in different size and type factories using different combinations of flour and methods of processing each possessing a different degree of exposure. None of them had adequate controls. An adequately controlled, comprehensive study might materially illuminate this subject.

The second situation to be explored is the epidemics of asthma caused by castor bean dust. This was selected to demonstrate a dual mechanism, one allergic the other toxic, that contributes to the production of asthma.

FIGLEY and ELROD [19] were the first to describe 85 instances of endemic asthma occurring within a radius of one mile of a plant that processed castor bean products. Some patients contracted asthma within their first year of residence in the locality while others lived in the area for as long as 17 years before developing it. Some of the victims failed to give positive cutaneous reactions to castor bean. ORDMAN [20] reported on an outbreak of asthma in Africa where over 200 employees or residents in close proximity to the factory were affected. As in Figley's

instance, the incidence of asthma bore a direct relationship to the degree of exposure, being highest during periods of greatest atmospheric contamination when the plant was operating and lowest or non-existent when the factory was idle or shut down. In Ordman's series there were 27 asthmatics who failed to demonstrate cutaneous sensitivity. Although the asthma was causally related to exposure to castor bean dust in all these cases, symptom production must have been by some mechanism other than sensitization in these 27 patients.

What proportion of all cases of asthma are caused by sensitization? This is extremely hazardous to estimate because most authors failed to use objective provocative tests to determine the clinical significance of cutaneous reactions. There are, however, a few very well controlled studies, that were designed to show the correlation between cutaneous and clinical sensitivity.

Information drawn from such studies might be applied to groups of asthmatics with positive cutaneous reactions and permit one to estimate the incidence of this type of asthma. HERXHEIMER [21, 22, 23], SCHILD [24] and COLLDAHL [25] have amply demonstrated the reliability and specificity of the reaction of the intact and isolated human asthmatic lung to the inhalation or addition of a specific allergen. They felt that the phenomena of histamine release and bronchial muscle contraction in allergic asthma was similar to that occurring in experimental anaphylaxis in animals. LOWELL and SCHILLER [26] reported on the diagnostic value of induced asthma indicating that clinically significant reactions occurred only to allergens that gave positive cutaneous reactions. Using similar techniques to positive reacting allergens in 512 asthmatic children, DAMGAARD [27] obtained 831 (43 %) positive reactions in 1933 trials. NILSSON and KAUDE [28] obtained significant responses to 1/3 of 1070 inhalation tests in a group of 200 asthmatics exhibiting positive skin reactions. Thus, somewhere between 33 and 43 % of all positive skin reactions are etiologically significant.

It now becomes necessary to determine the incidence of cutaneous reactivity in large groups of asthmatics. The second table indicates the percentages of cutaneous reactions in some of the larger group studies.

TABLE 2

Incidence of cutaneous reactions in asthma

Author	Number of Patients	Incidence in %
Alexander (29)	4809	52.7
Cooke (30)	688	50.2
Frugoni and Serafini (31)	4400	44.5
Spouyetch et al. (32)	2238	60.0
Diaz (33)	7628	24
Vaery-Radot (34)	803	53.0
	20566	8443

Averaging these figures out indicates that 8443 of 20655 (41 %) of asthmatic subjects display positive cutaneous reactions. If from 33 to 43 % of these are etiologically significant it can be estimated that from 13.5 to 17.6 % of all asthmatics are due primarily to sensitization.

Classification based on etiological factors are untenable for they assume that reactions to exogenous or endogenous agents are mutually exclusive, when in reality, the same patient generally reacts to both, singly or in combination; at one time or another. Some allergists also feel that sensitization is a necessary prerequisite to the development of asthma, a theory that lacks substantiation and one with which we find ourselves in disagreement.

There is more or less universal acceptance of the fact that when sensitization exists in asthma of infancy and early childhood, it is generally due to foods. RATNER [35] found food allergy in 100 % of his 27 asthmatics during their first year of life. This levelled off to 60 % by the fifth year. SPAIN [36], likewise, recorded its importance up to age 20 with diminishing or negligible importance as a cause of asthma thereafter. Among the principal food offenders he listed eggs, milk, seafood and chocolate. In adult asthma caused by sensitization there is also unanimity of opinion regarding the major role played by house dust, feathers, various danders, pollens and molds. The actual percentages cited by investigators reflect, to a great degree, the environmental exposures in each instance. Thus, DAMGAARD [27] found 73 % reacting to horse dander, while VALLERY-RADOT [34] found a minority (10 %) reacting to animal emanations. Thus, it can be concluded that the highest percentage of sensitization in asthma occurs in the younger age group and decreases with advancing age. As demonstrated by PEARSON [37] such cases cause less disability, have a higher percentage of remissions, rarely have a fatal outcome and respond better to treatment.

DISCUSSION.

The problem of sensitization in asthma has been discussed both from the standpoint of the mechanics of sensitization and from the role of the subject. The manner in which the allergic state was produced was reviewed in depth for it offers the investigator the only reproducible means of determining the mechanics of symptom production and gives him a model for devising ways and means of combating them. Some of the deviations, qualitative and quantitative, were explored without attributing them to a specific cause or trying to assign to them any special significance.

From the clinical standpoint one gains the impression that the allergic asthma occurs most often along direct family lines. Despite the remarkable advances made in our understanding of the immune processes in the last decade or so, little or no progress has occurred in clarifying why sensitization provokes symptoms in some individuals and not in others. Sensitization, per se, does not seem to be based on

the possession of any special type predisposition for it occurred with equal frequency in normal nonatopic controls. Yet there must be some basis to explain why asthma of this type occurs more often in the children of those suffering with it. Ample corroboration for such can be found in the numerous population studies bearing on this question. Although science cannot presently explain if and where such tendencies exist or how they are transmitted, sufficient clinical observations have been cited to indicate that some differences do exist. Whether these factors are the actual basis for such selection or not awaits scientific substantiation. This seeming paradox will be settled only by adequately controlled clinical studies and by a better understanding of the manner by which allergic traits are genetically transmitted, if indeed such is the case.

Attention was called to the fact that asthma often follows in the wake of an allergic rhinitis. In Linko's [17] study only 6 of the 63 exhibited asthma without an associated rhinitis. Likewise, in our own study [18] on nasal polyposis, a large proportion of the patients (72 %) ultimately developed asthma. It has been estimated that between 25 and 50 % of hay fever victims eventually develop asthma. Allergic asthma then may be considered the end result or the most advanced state of respiratory sensitization. The time to counteract progression is before it has reached its ultimate destination.

What checks and balances has nature endowed us with that accounts for selection and localization of the effector organ? Part of this may be due to the special nature of the allergen and its portal of entry. Thus, the molds seem to evoke a primary bronchitic reaction while provocative challenge to pollens is invariably manifested by and limited to the upper respiratory tract. Flour, on the other hand, may involve the skin as well as the upper and lower respiratory tract. Local irritation of the respiratory tract with smoke and chemicals prior to exposure to allergens did not enhance sensitization. Clinically, irritants of this type frequently precipitate symptoms in otherwise quiescent asthmatics. That asthma is not solely the result of sensitization is pointed out by citing examples of asthma following exposure to castor bean dust [19, 20] and infected grain [39, 40] without concomitant sensitization. Cognizance must be taken of substances that are modified by contaminants. The mechanism of symptom production due to each must be ascertained despite the fact that the clinical management may be the same in all instances. By so doing one often separates the sensitizing potential of a substance from its asthmogenic capacity.

A large proportion of sensitized individuals never manifest clinical allergies, so-called latent sensitivity. What initiates effector organ participation or renders it refractory to further allergenic stimulation? Surely, all known immunological parameters remain constant before, after and while active allergic disease is present. Provocative testing with micronized or aerosolized allergen is an accurate method of judging the reactivity of the pulmonary tissues.

Finally, the fallacy of trying to classify asthma in accordance with

the categories of agents that provoke it is pointed out. The etiologic pattern is different qualitatively and/or quantitatively in each case of asthma. Generally the substances provoking asthmatic symptoms may vary from one attack to another and cross all arbitrary classifications. If asthma is one of the primary responses of the respiratory tract then sensitization is only one of its causes and albeit not the most important. It does hold a key that the others lack, that of reproducibility, that might lead to the unlocking of the many poorly undestood mechanisms that are associated with it.

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ALLERGIC AND NON-ALLERGIC FACTORS IN THE AETIOLOGY OF BRONCHIAL ASTHMA

H. HERXHEIMER.

Although most diseases have a multifactorial aetiology, we may regard, in some cases, a single factor as the principal or main cause of the disorder, as, for instance, the vibrio cholerae in the case of cholera. But even here subsidiary factors exist, for example, the immunological state of the subject and its genetic disposition. If such other factors did not exist, every infection with the vibrio would cause exactly the same degree of disease.

In asthma, the aetiology is more complicated. There is, first, the genetic factor which we call the inclination of the subject to develop bronchial spasm. Independent of this are external factors which may be allergic or non-allergic. The allergic factor consists, as we know, of the formation of antibody in certain cells —target cells— and its contact with antigen. This contact leads in a very complicated way to the formation of histamine, slow reacting substance and probably a number of other substances in these cells. As the place of contact is the mucus membrane with its autonomic ganglia and the bronchial muscle close to it, overproduction of bronchial mucus and bronchial spasm is the result. If this mechanism operates numerous times and the nervous structures involved are stimulated every time, it is possible that these structures will respond to stimuli other than those caused by the antigen-antibody reaction. There is indeed evidence that this happens. If one sensitizes guinea-pigs against egg albumen and exposes them to the aerosol of the antigen at regular intervals, one sometimes can see animals which develop the typical dyspnoea of anaphylactic shock when all the usual external conditions of exposure to the antigen are fulfilled except the presence of the antigen. NOELPP and NOELPP-ESCHENHAGEN (1952) have found that this effect which is based on the development of a conditioned reflex can be reproduced at will. If this is possible in guinea-pigs, it is not unlikely that similar conditions may occur in man. There are many well known observations that in some subjects certain visual or olfactory stimuli which have been previously experienced in allergic attacks, can start an allergic reaction without the antigen being present. In other cases anxiety alone has been known to cause an allergic reaction.

It is, however, doubtful whether such a reaction can be produced without a previous increase in the irritability of the bronchial tree.

This irritability may have increased in consequence of numerous antigen-antibody explosions or by local irritation through infection. We know of many patients in whom the asthma has started in immediate connection with an upper respiratory infection. Many investigators have tried to trace these cases of asthma to allergy to certain bacteria. But to my mind, evidence for such allergy has not been found, and the recent results of HAMPTON (1963) have not altered this position. Also, the therapeutic evaluation of the theory of bacterial allergy has not been encouraging. So far, clear cut therapeutic success has been missing, and such successes as have been reported may have been due to unspecific desensitization.

We have to deal, therefore, with 2 groups of primary increase of bronchial irritability for which a genetic disposition is necessary. One is the allergic group in which the antigen-antibody reaction is the causal factor, and the other is inflammation or infection. This group is usually labelled infective or intrinsic, but this label means nothing else but that we cannot find any allergic aetiology. In their symptoms these asthmatic patients are very much like chronic bronchitics, with the only difference that they have, apart from their overproduction of mucus, a higher degree of bronchial spasm. As ordinary chronic bronchitis also shows some degree of bronchial spasm (as can be seen if isoprenaline is given in a closed spirometer circuit) there is only one small difference between both conditions, namely the greater inclination of the asthmatic to bronchial spasm shown by apparently spontaneous attacks. It almost looks as if the difference between chronic bronchitis and infective asthma is the genetic disposition of the latter. If this is correct, we would expect that an acute upper respiratory infection would heal up without any consequences in a normal subject, whereas in a subject with asthmatic disposition it may induce asthma. In that intermediate group in which the bronchitis does not disappear but becomes chronic we must suspect that some tendency for increased bronchial irritability has existed, but not enough to cause typical asthma. This assumption is strengthened by our finding (HERXHEIMER & STRESEMANN, 1961) that chronic bronchitics usually have sputum eosinophilia, as have typical asthmatics.

If the further development of these patients is followed, the ultimate result often will be emphysema with bronchitis. These patients also have, in many cases, pronounced sputum eosinophilia. Moreover, in the later stages of the disease, they rarely can be distinguished from chronic asthma. Their symptoms and signs are very similar, and, particularly, the bronchial spasm, which, in the beginning of the disorder played second role to hypersecretion becomes more prevalent in the later stages and the nocturnal attacks so typical of genuine asthma are added to the symptoms. Clinically these patients who originally had no typical asthma now cannot be distinguished from it, although, at necropsy, they will show emphysematous changes which are absent in many pure asthmatics, as GOUGH (1955) has showed.

If this working hypothesis about the relationship of bronchial

asthma and chronic bronchitis is correct, experimental proof for it must be sought. Perhaps it will be possible to cause chronic bronchitis in an animal species which is also subject to asthma. The unitarian view of asthma and bronchitis would explain many features common to both disorders: Once irritability of the bronchial tree has developed, many so-called causes could be held to be superimposed stimuli only. Hyperventilation with excess loss of CO_2 (hyperventilation asthma, HERXHEIMER, 1946), irritant gases (SO_2) and, most important, psychological influences would fall into this group. As several of them may occur together, their practical importance is great.

The tentative explanation I have put forward has the advantage that it is compatible with clinical experience. It remains to be seen whether experimental proof is possible.

To sum up: Bronchial Asthma is caused by inclination of the bronchial muscle to develop irritability. This irritability can be acquired by repeated contact with allergens or by chronic inflammation of the mucous membranes. Once the irritability has been established, many factors can elicit bronchial spasm and hypersecretion, for instance irritant gases, loss of carbon dioxide through hyperventilation and central nervous impulses.

THE PATHOLOGICAL ANATOMY OF ASTHMA CONTRASTED WITH THAT OF CHRONIC BRONCHITIS

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Many clinicians in Britain separate chronic bronchitis from asthma as distinct diseases (Ciba Symposium, 1959). In some other countries the two conditions are regarded as variants of the same process. Indeed it may be asked whether anything is to be gained in trying to separate the conditions. A considerable proportion of those who suffer from chronic bronchitis develop "asthmatic" symptoms and many of those who start with a condition of intermittent bronchospasms end up with bronchial infection. Thus the two conditions may develop to a stage where they are indistinguishable. There are those who regard all cases of bronchitis as due primarily to the individual's constitution and believe that allergy or hyperreactivity predisposes to the harmful effects of environment in susceptible individuals (ORIE et al, 1961). In Britain however, we are inclined to take a different view. We recognise a condition asthma based on constitutional allergy but the much more common condition of chronic bronchitis is regarded as due to environmental conditions for the following reasons. Chronic bronchitis is much more common in industrial than in rural districts; it is related to cigarette smoking; it has a greater frequency in men than in women; it is fifty times as frequent in some occupations as in others; the lower the social class the greater the chance of death from chronic bronchitis; the incidence is rising and now has a mortality of 30,000 per annum in England and Wales.

Part of the claim that host factors are prime in all chronic "bronchial" disease is based upon sputum eosinophilia. The incidence of eosinophiles however has been found to be much lower in chronic bronchial diseases in Britain than for instance in Holland. There is a marked contrast in the findings in these two countries. Eosinophilic bronchitis which MULDER (1956) described as the commonest form of chronic bronchitis in Holland is seemingly rare in Britain. ORIE et al (1961), also in Holland, found more than 60 % eosinophiles in the sputum in 27 out of 35 bronchitic patients. It seems that they are dealing with a disease different from that described by MILLER (1963) of London. In his series

of bronchitics only 2 out of 394 had more than 60 % eosinophiles. Neither of these two men was asthmatic.

HERS (1961) of Holland has also emphasised the importance of eosinophiles and the majority of this patients with bronchitis had sputum eosinophilia. In Britain eosinophiles are found in patients with asthma but hitherto they have only been found in a low percentage of patients with chronic bronchitis. Using Hers methods on male factory and office workers in London, MILLER found a higher degree of sputum eosinophilia than had been previously noted in British chronic bronchitis but nevertheless 60 % of the patients had few or no eosinophiles. Using the criteria of ORIE et al, eosinophilic bronchitis would seem to be rare in Britain and in contrast there is a high incidence of British bronchitis with few or no eosinophiles.

HERXHEIMER and STRESEMANN (1961) in Berlin found sputum eosinophilia in 93 % of 120 cases of typical asthma and also in 8 out of 9 instances of chronic bronchitis without dyspnoea. In 61 patients with typical emphysema in whom unprovoked attacks of dyspnoea did not occur or occurred only in the later stages of the disease, they found that 70 % had sputum eosinophilia. CHODOSH et al (1962) in the U. S. A., on the other hand, examined the sputum from 21 patients with long standing chronic bronchitis. Neutrophiles were the predominant cells and eosinophiles constituted a very small part of the cellular response.

To obtain further information on the incidence and importance of eosinophiles, Dr. J. G. LEOPOLD, working on cases collected in my department, has estimated the percentage of eosinophiles and other cells in the lungs of individuals who had died of chronic pulmonary disease which had been diagnosed either as chronic bronchitis or asthma. There were 60 cases many of whom had been patients of Dr. D. A. WILLIAMS. 35 of these had been diagnosed clinically as asthma and had died in an attack of status asthmaticus. The remaining 25 had been variously diagnosed, some as asthmatics, some as chronic bronchitis and some as chronic bronchitis with asthma. Eosinophiles, mast cells, polymorphonuclear leucocytes, lymphocytes and plasma cells were counted and were then represented as a percentage of all the cells present and also in absolute numbers of cells per unit of the bronchial tissue, cartilage being excluded. Striking results were obtained in the values of eosinophile cells. The percentage and the absolute numbers of eosinophile cells were high in cases dying in status asthmaticus and with a single exception were completely different from the values obtained in the other cases. In the status asthmaticus cases the eosinophile percentages ranged from 7 to 40 %. The other cases had less than 4 % and the majority less than 1 %. There were similar findings in the absolute numbers of eosinophiles present. In the status asthmaticus cases these varied between 10.3 and 441.6 per sq. mm. whereas in the other cases they were less than 9.6 per sq. mm. of tissue and in most of them less than 3.2. In the group which had not died of status asthmaticus there was no difference in the eosinophile count whether they had been regarded as asthmatic or chronic bronchitis. The two cases which stand intermediate between

the groups died of status asthmaticus and had much lower eosinophile counts than the other 33. One had 4.5 % and the other one had 1.2 %.

These findings support the view previously held in Britain that the cell response in asthma is different from that in the much more common chronic bronchitis.

Most of the cases which show high eosinophilia in the lung at autopsy are those which had a history of asthma and died in status asthmaticus.

The bronchi showed the characteristic mucus plugging. The mucus is continuous from the lumen to the interior of the cells, which accounts for the difficulty in expulsion and for the stripping of the epithelium that occurs.

Asthma and Emphysema.

For many years pathologists have noted that asthma may not lead to the development of emphysema. LOESCHCKE (1928) reported several such cases. RACKEMANN (1944) described the autopsy findings in 50 patients dead of asthma but structural emphysema was found in only two. The characteristic plugs of mucus were present and there was dilatation of the alveoli, but such dilatation was referred to as being functional by which one can suppose the authors meant reversible. STRESEMANN (1962) reported the absence of destructive emphysema in a 37 year old housewife who had asthma for 10 years and had been seventeen times in hospital with status asthmaticus. DUNNILL of Oxford (1960) examined 20 cases of death due to status asthmaticus. He found no destructive emphysema and agreed with me (GOUGH, 1955) that the lungs were overdistended as in drowning. On the other hand GAY (1946) in the U. S. A. recorded destructive emphysema in 23 out of 24 patients in whom asthma was regarded either as the primary or secondary cause of death. This experience is quite unlike that of Rackemann, Dunnill and myself. 13 of Gay's patients showed hypertrophy and dilatation of the right side of the heart and in 62.5 % of his cases he regarded heart disease as playing a major part in the death. Of 20 patients examined 60 % had less than 3 % eosinophiles in the blood. Purulent bronchitis and pneumonia was the primary cause of death in 15 and a further 3 died of bronchitis after morphine administration. The findings are what I would expect in cases of chronic bronchitis rather than in allergic asthma.

Gay's findings are in contrast to a series of cases from my department reported by WILLIAMS and LEOPOLD (1959). Destructive emphysema was not found in any of the 19 women in a series of 25 but occurred in two men in the series. Right heart hypertrophy was found in 5 of the 25. There is difficulty in comparing different series of cases because ballooning of the lung due to bronchial obstruction may be reported as emphysema and such ballooning is usually present. Destructive emphysema however is not a prominent feature and may be absent (GOUGH, 1961). The presence of emphysema can readily be seen in whole lung sections (GOUGH and WENTWORTH, 1959-1960) and there is marked contrast

between destructive centrilobular and panacinar emphysema in chronic bronchitis and simple overdistention in asthma. The accurate diagnosis of emphysema requires special preparation of the lungs particularly fixation in expansion. The difference in the incidence of destructive emphysema in asthma and in chronic bronchitis may be due to the difference in the situation of the lesions. In bronchitis inflammation extends to the bronchioles and then to the respiratory bronchioles and often into the alveolar walls. In asthma however the inflammation tends to stop proximal to the respiratory bronchioles and only occasionally does the eosinophilic kind of inflammation extend into the parenchyma of the lung. When destructive emphysema occurs in asthma it would seem probable that it is due to the same causes that produce chronic bronchitis, i. e. to the additional effects of smoking, general atmospheric pollution and infection. In asthmatic women it is rare to find destructive emphysema even when asthma has been present for many years. In asthmatic men however destructive emphysema is more likely to be found. Men are more likely to be smokers and the emphysema found is of the centrilobular and the panacinar types associated with chronic bronchitis and cigarette smoking seems to be at least one important cause.

Sub-segmental areas of bronchiectasis are not unusual in asthma. In most instances they appear to result from atelectasis, probably in previous asthmatic attacks, although occasionally, as in middle lobe bronchiectasis, they may have been the trigger for the asthma. In chronic bronchitis the bronchi show more diffuse or cylindrical dilatation, a feature which is well seen in whole lung sections.

SUMMARY.

Fatal asthma has a distinctive pathology characterised by eosinophilic infiltration, mucus plugging of the bronchi and overdistention of the air spaces. In contrast, chronic bronchitis has little or no eosinophilic infiltration, destructive emphysema is common and death is from cor pulmonale or respiratory failure or pneumonia.

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AEROBIOLOGY AND BRONCHIAL ASTHMA

P. J. VAN DER WERFF.

The Netherlands.

CADHAM, STORM VAN LEEUWEN, JIMÉNEZ DÍAZ and HANSEN were the first to demonstrate that airborne fungusspores can be responsible for symptoms of respiratory allergy. Aerobiological investigations can be carried out from the point of view of the biologist, entomologist, phytopathologist, hygienist or allergologist, each of whom makes his own demands.

For the allergologist and his co-workers, it is of importance to obtain a sufficient number of reliable data for clinical work; this implies the numbers and types of anemophiles particles, identified as exactly as possible, that is down to species level, their regional, annual seasonal and diurnal occurrence in the outer atmosphere, their occurrence in the air, indoors.

The fundamental lines of three methods of aerobiological investigation are established. A comparison is made between their different sites in the Netherlands, airborne spores were studied during 1959, 1960, 1961.

The aerobiological results will be presented at the Congress and the clinical importance of airspora will be discussed.

PULMONARY MECHANICS, GAS EXCHANGES, AND CIRCULATION DURING ACUTE BOUTS OF ASTHMA AND IN INTERVENING PERIODS

A. COURNAND.

New York

The dominant structural anomaly of allergic acute bronchial asthma consists of a reversible obstruction of the smaller bronchi and non respiratory bronchioles by mucous secretions and sub-mucosal edema, *of variable intensity and extent.* This obstruction, associated with a more or less severe degree of constriction of the smooth muscles of the smaller airways, leads to a diversity of interrelated physiological disturbances. The physiological disturbances, varying with the intensity and extent of the structural and functional obstruction, command in turn the severity of the clinical manifestations.

The physiological disturbances may be classified as: 1. Mechanical, upon which depends the degree of dyspnea; 2. distributional, with regard to regional ventilation and blood flow, upon the relationship of which depends the appearance of anomalous respiratory gas exchanges (arterial blood hypoxemia, acute respiratory alkalosis or acidosis) and of their effects on the pulmonary vascular resistance.

In our presentation we shall successively deal with: I. The well established physiological disturbances; II. The results of recent studies based on new techniques or new concepts which have led to a better understanding of the physiopathology of bronchial asthma; III. The problem of reversibility of physiological disturbances between and following acute attacks of asthma.

I. CLASSICAL PHYSIOPATHOLOGICAL MANIFESTATIONS OF ACUTE BRONCHIAL ASTHMA.

These have been described in detail during the past 30 years and particularly well discussed by ROSSIER, BÜHLMANN and WIESINGER in their monograph on *Respiration.*

A. *Mechanical Disturbances*:

(1) The chest cage assumes a deep inspiratory position (reflexly?) as an effect of airway obstruction. This is accompanied by an increase in the force of retraction of the lung structures. It leads to a temporary

increase in the functional residual capacity (F. R. C.) and residual volume demonstrable by measurement during the acute phase. (2) The air flow resistance is increased-not only in the airways obstructed, but outside these areas by the effects of air trapping upon non-cartilagenous portions of bronchi (large or small); the air trapping resulting from expiratory check valve mechanism; (3) The tissue deformability resistance, normally dependent on air flow velocity, cannot be estimated with accuracy in asthma since local alterations of lung volume interfere with measurements of airway resistance; (4) The dynamic lung compliance (elasticity), normally dependent on lung volume, becomes less as a result of regional variations in air flow velocity.

The resistance to airflow related as indicated above, to "obstacles" within the lumen of or outside the airways, varies with these, and is mostly expiratory, but also demonstrable in inspiration. It increases the work of breathing, and therefore the oxygen cost of breathing, to sometimes more than 10 times their normal values. Airway obstruction of moderate degree leads to hyperpnea, but as it becomes more severe and extensively distributed, bradypnea may supervene. The subjective feeling of dyspnea is intimately related with these various factors.

Simple spirometric tests may help demonstrate the severity of obstruction and of its effects upon the mechanics of breathing: variable reduction of vital capacity, of maximum breathing and of the several indices associated with Timed Vital capacity. These tests may also be used to demonstrate the effects of drugs, and the degree of reversibility of the obstructive syndrome.

B. *Disturbances of air and blood distribution.*

It is now well understood that disturbed relations between local alveolar ventilation and blood perfusion play a major role in the appearance of arterial blood hypoxemia and acute respiratory alkalosis or acidosis. The presence and severity of arterial hypoxemia depend predominantly upon the degree of regional hypoventilation of alveoli still well perfused, leading to reduction in oxygen partial pressure. The effects of alveolar hypoventilation of some alveolar units upon the arterial blood O_2 saturation cannot be compensated for by hyperventilation of other areas; in contrast, the overall partial pressure of carbon dioxide in the arterial blood (low or high, with associated acute alkalosis or acidosis) is controlled by the magnitude of alveolar hyperventilation in regions in which the airways are not obstructed.

These general notions explain the diversity of reported observations concerning the influence of acute asthma upon the state of arterial blood respiratory gases (absent, mild, severe hypoxemia, respiratory alkalosis and acidosis).

Since local alveolar hypoxia increases the pulmonary vascular resistance to blood flow (probably by vasoconstriction of the smooth muscles of the small arteries), the presence and degree of pulmonary arterial hypertensión will depend, as suggested by the experiments on induced

asthma in humans reported by SCHERRER et al., upon the extent and severity of alveolar hypoventilation.

BATES et al., WILLIAMS et al., have interpreted the normal values of diffusing capacity of the lung, usually measured using one of the carbon monoxide techniques, as indicating that the size of the pulmonary capillary bed is not encroached upon, and that the alveolar capillary interphase remains normal. However, interpretation *of the results* of measurements of diffusing capacity, in the presence of disturbances of alveolar ventilation/perfusion relationship is of questionable validity.

II. RECENT STUDIES CONCERNING THE PHYSIOPATHOLOGY OF ACUTE REVERSIBLE BRONCHIAL ASTHMA.

A. *Mechanics of Air Flow.*

Using the technique of Fry and Hyatt, G. P. Lord, and Alfred P. Fishman, have studied in acute asthma the forces involved in the movement of air between alveoli and ambient air.

The technique requires catheterization of the esophagus, a pneumotachygraph, an integrator and an oscilloscope. This permits the simultaneous recording of instantaneous rate of air flow, air volume changes within the lung and the transpulmonary air pressure differential (esophagus to mouth). The flow-volume and flow-pressure loops simultaneously recorded may then be analyzed at any instant of the breathing cycle.

In patients during acute asthmatic attaks, they found: (a) the rate of air flow to be low throughout all of the expiration, and (b) to decrease markedly as expiration is prolonged, even though intrathoracic pressure continues to increase. In contrast to performance in the normals therefore, *air flow is dependent upon lung volume and not driving pressure*, even at the beginning of expiration. This is attributable to the caliber of airways at the height of inspiration. In normals at this moment the airway caliber is large, leading to a very low airway resistance and consequent early peak expiratory air flow with marked differential pressure drops. On the contrary in patients with acute asthma the caliber of the airways is markedly restricted even at the height of inspiration.

In contrast to observations made in chronic obstructive diseases of the lung, there is no sharp drop in air flow with high driving pressures (above 15-20 cm H_2O); this difference suggests that the bronchi are less apt to collapse in asthma, and is well supported by anatomical observations: airsways are thick-walled with edema and smooth muscle hypertrophy in asthma, instead of being weak-walled and flail as in emphysema.

Inhalation of bronchodilator drugs during an acute attack leads to an increase in the rate of air flow at the end of expiration, but this rate is still dependent upon lung volume during the entire cycle. These findings are consistent with pathological and clinical evidence that acute asthma affects predominantly the smaller airways.

After full clinical recovery, the maximum flow rate becomes dependent on the driving pressure at higher lung volume (beginning of expiration) as is the case in normals.

B. *Distribution of Tidal volume in the lung.*

Recently Gomez, of the Columbia University Cardio-Pulmonary Laboratory at Bellevue Hospital, has proposed a new concept and a new method for the analysis of inert gas pulmonary washout function curves. These may be interpreted either in terms (a) of continuous distribution function curves of specific tidal volume $\frac{V_t}{V_0}$ where number of lung units is plotted against ratios of tidal volume change per unit volume, or (b) of an *apparent volume* calculated from such distribution curve; this apparent volume in the normal is equal to the measured functional residual capacity (F. R. C.). The ratio of the apparent to the measured can be taken as an index of effective ventilation.

In young children studied with Juan Filler during and following recovery from acute attacks of asthma, it has been found that: 1) the distribution curve of effective tidal volume is considerably different from normal distribution curves: more particularly a considerable number of lung units show a very slow turnover rate, and returns to a normal form following the attack, and 2) the apparent volume may during the attacks be calculated as three times greater than the F. R. C., indicating a considerable reduction of effective ventilation in large areas of the lungs.

C. *Role of blood hydrogen ion concentration in the control of pulmonary vaso-activity.*

The role of blood hydrogen ion concentration has been studied in relation to pulmonary hypoxia, by Enson, Harvey, Ferrer, in the same laboratory. Evidence is forthcoming that the local effect of alveolar hypoxia upon the pulmonary vascular resistance can be partly or totally inhibited by blood alkalosis, and markedly enhanced by blood acidosis. Hydrogen ion concentrations rather than CO_2 partial pressure is involved in this interrelated effect upon the tone of the musculature of the pulmonary vessels. The interpretation of results of measurements of pulmonary arterial pressure in the course of acute attacks of asthma should greatly benefit from this new development.

III. Problem of complete reversibility of physiological disturbances in allergic asthma.

Pediatricians with large experience in allergic asthma have emphasized the very rare occurrence, after acute attacks have subsided, of developing chronic obstructive disease of the lungs. On the other hand, manifestations of chronic obstructive disease of the lungs with mani-

festations of cor pulmonale, may occasionally be observed among adults with a long history of asthma, sometimes dating back to childhood. In view of this apparent paradox, recent investigations with refined physiopathological methods are worth discussing. They have shown that persistance of functional disturbances in clinically healed and symptom-free patients is not unusual.

(1) LORD and FISHMAN have demonstrated in the asymptomatic asthmatic with the method previously referred to, (a) abnormal flow volume and flow pressure curves, and (b) a considerable increase of work in tidal breathing. Furthermore, abnormalities of flow volume curves have also been recognized in some patients months after recovery from an acute attack.

(2) D. BATES, B. ROSE and their collaborators at MCGILL University have recently studied in adult patients during remissions between acute attacks the regional distribution of pulmonary ventilation and perfusion using $Xenon^{133}$ as a tracer. With this technique the course of gas distribution is followed: (a) after inhalation of a mixture containing the tracer gas, during equilibration with a closed circuit, and (b) after intravenous injections of the tracer gas in solution. The gamma radiations emitted by the inhaled or injected $Xenon^{133}$ are counted by properly placed scintillation counters. In 6 of 12 patients some impairment in distribution of the inhaled gas was demonstrated to be zonal rather than diffuse, whereas the distribution of blood flow appeared normal. Such a residual ventilatory disturbance corresponded to other minor defects revealed by more conventional function tests: reduction in helium mixing efficiency, of forced expiratory volumen and of maximal expiratory flow; in all the 12 cases the steady resting diffusing capacity of the lung for carbon monoxide was measured at normal level.

Future work in the physiopathology of asthma should obviously be oriented towards the nature of the "obstacles" which persist during remission or apparent health. This may possibly help to understand the mechanisms involved in the development of chronic obstructive disease of the lungs in patients with chronic allergic asthma.

THE EFFECT OF BRONCHIAL ASTHMA ON BLOOD GASES, BLOOD ELECTROLYTES, ACID BASE BALANCE AND BODY TISSUES

H. COLLDAHL. (*)

There are at present different opinions concerning what ought to be called bronchial asthma and what chronic bronchitis and what emphysema. In this study asthma is considered to be present in patients with more or less reversible stenotic breathing of expiratory type caused by diffuse changes in the small bronchi. In my opinion bronchitis should be reserved for an infectious process in the bronchi. Further in case of stenotic breathing it is important to distinguish between the following conditions:

1. Without destruction of lung tissue.
   - A. Without primary infection.
     - a. Allergic bronchopathia.
     - b. Unspecific bronchopathia.
   - B. With primary infection.
     - a. Acute.
     - b. Chronic.

2. With destruction of lung tissue.
   Emphysema.

In all of the above mentioned conditions hypersensitivity and infection may have importance for the actual symptoms.

1. *Blood gases.*

MEAKINS (1924) was the first to show that severe attacks of spontaneously occurring asthma were associated with an increase in the carbon dioxide tension and a decrease in the oxygen saturation. In mild attacks, however, no carbon dioxide retention was found but a moderate and inconstant oxygen desaturation.

These findings have later been verified both in experimental animals and in patients with asthma by many investigations (DANTREBAUDE,

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1934; COLLDAHL, 1941-1943, 1947; ROSSIER, 1943, 1950-1951; COLLDAHL and LUNDIN, 1952; BALDWIN, 1946; SCHILLER and collaborators, 1951; SCHERRER and collaborators, 1956, and TURIAF and collaborators, 1962).

COLLDAHL (1941, 1942, 1943, 1947) showed that in experimental asthma on guinea pigs the pulmonary ventilation and oxygen uptake are altered in an entirely regular manner though differently depending on the severity of the asthma. In severe asthma the pulmonary ventilation and oxygen uptake are considerably less than normal, while in rather mild attacks both are raised in relation to the normal. After a severe asthmatic paroxysm the oxygen uptake and pulmonary ventilation are substantially above normal, which shows that there has been an oxygen deficit during the attack, a deficit that is compensated for after the attack is over. This was a regular finding, and it is suggested that the inconsistent reports in the literature concerning pulmonary ventilation and oxygen uptake probably have their cause in the determinations having been carried out at different points of time in relation to the asthmatic attacks and that these attacks have been unequal in severity. The oxygen saturation of the arterial blood is lowered during rather severe attacks, and the lactic acid content of the blood is simultaneously raised, which latter finding can be interpreted as suggesting that there was a lack of oxygen in the tissues. The impaired interchange of gases resulting from the stenosis of the air-passages in severe asthma is considered to be the primary cause of the changes set forth above. A disturbance of the intermediate metabolism was found to be present.

COLLDAHL, 1947, showed that similar changes occur in man in spontaneous attacks of asthma as far as lung ventilation and oxygen uptake are concerned.

COLLDAHL and LUNDIN, 1952, showed that in patients with bronchial asthma the ventilatory changes in even the mildest forms of provoked asthma can be objectively registered with a minimum of active co-operation by the patient by measuring the time required for the patient to wash out the nitrogen from the lungs on resting breathing after the transition from air to oxygen breathing. The nitrogen washing out is followed by a continuous registration of the nitrogen content of the alveolar air by the help of a so-called nitrogen analyser. In addition to being used in provocation tests the method has been used by the authors for studying the course of the disease in asthmatic subjects.

In these provocation tests with only slight asthmatic changes no fall in the oxygen saturation of the haemoglobin appeared as measured by the Millikan oxymeter.

SHERRER (1956) and his collaborators found in experiments with induced asthmatic attacks (by an allergen or 3 % histamine) that all types of abnormalities may develop in the arterial blood gas composition. Sometimes a respiratory alkalosis due to alveolar hyperventilation was found. In other cases a slight hypoxemia appeared while occasionally severe respiratory acidosis developed (acute alveolar hypoventilation).

According to SHERRER the hypoxemia is caused by an extensive per-

TABLE

(Rossier, Bühlman

	P. Z.	H.
	During attack	During attack
Arterial blood		
O_2 saturation %	92 (95-97)	81
O_2 tension mm. Hg	62 (85-95)	48
CO^2 content vol. %	37.3 (54-57)	51.6
pH	7.46 (7.38-7.41)	7.41
CO^2 tension mm. Hg	23.2 (40)	35.6
Spirometry		
Resp. quotient	0.82 (0.82)	0.70 (0.82)
Minute volume ml/min.	23490 (6830)	13900
Alveolar function		
Alveolar vent.	7440 (4320)	6300

Predicted normal values in brackets. The patients P. Z. (35 year-old-male), H. ST. (33 diagnosis: Bronchial asthma. Patient K. B. (51 year-old-male)had chronic bronchial ast

fusion of alveoli with trapped air, causing a high venous admixture effect.

SHERRER concludes (1956) that extra pulmonary factors as well as primary lung rigidity can according to his investigation be excluded as important factors in asthmatic dyspnoea.

On the other hand HANSEN and SCHLEINZER (1949-56) have a divergent viewpoint on this question as they believed that in provoked asthma a decrease in oxygen saturation of the arterial blood takes place before any difficulty in breathing is experienced by the patient. COLLDAHL (1959) discusses this problem and comes to the conclusion that the change in oxygen saturation depends on pulmonary and cardiovascular changes.

Chronic alveolar hypoventilation is considered to exist when the carbon dioxide tension is increased to 45 mm Hg or more and remains at approximately the resting levels during exercise (ROSSIER, BÜHLMAN-WIESINGER, 1960). These authors give examples of different data for blood gases, pH, spirometry and alveolar function in asthmatic patients with different degrees of asthma.

If the stenotic process is slight, significant alveolar hyperventilation with respiratory alkalosis is frequently observed. If the stenotic processes are fairly severe, an increase in alveolar hypoventilation develops. The arterial oxygen tension decreases and the CO_2-tension increases. This is seen clearly from table 1, patient P. B. (ROSSIER, BÜHLMAN, WIESINGER).

I

Wicsinger, 1960)

ST.	M. T.	P. B.		K. B.
After broncho-dilatation	During attack	During remission	During attack	Rest room air
89.4	89.7	92.8	45.5	81.7
55	65	68	28	51
46.1	50.8	50.4	75.2	74.7
7.46	7.27	7.42	7.26	7.32
28.5	47.6	34.1	72.1	63.3
0.72	0.92 (0.82)	0.89 (0.82)	0.75	0.82 (0.82)
12800	8250 (5740)	7820	14890	9470 (6490)
8780	3440 (4100)	5060	3230	2590 (4110)

year-old-female), M. T. (47 year-old-old-female) and P. B. (16 year-old-male) had the hma.

Patients P. Z. and H. ST. show a decrease in oxygen saturation, despite hyperventilation and a marked increase in residual volume. Patient M. T. has an acute alveolar hypoventilation. Patient P. B. developed severe alveolar hypoventilation during the attack. The arterial oxygen saturation is very low.

TURIAF and collaborators have 1962 investigated blood-gases and acid-base balance in patients with bronchial asthma. They have found different degrees of respiratory alkalosis and acidosis in different forms of bronchial asthma. The oxygen saturation was decreased in most cases, but the intensity and frequency of the desaturation was different for the different forms. (See table 2.)

HELANDER, LINDELL, SÖDERHOLM and WESTLING (1962) have shown that a moderate to severe asthmatic attack induced by a specific allergen was associated with a small increase in pulmonary artery pressure and a tendency towards an increase in pulmonary vascular resistance. They have further shown that the effects of inhaled allergen and inhaled histamine on the pulmonary ventilation and circulation were indistinguishable. The injection of histamine did not cause any asthmatic attacks, but nevertheless the arterial oxygen saturation fell (more than during inhalation of histamine) and the pulmonary vascular resistance decreased.

How the blood gases change in asthmatic patients during work has been studied by COLLDAHL and HOLMGREN:

From table 6 it is found that the O_2-tension in arterial blood in rest

TABLE 2

(Turiaf and collaborators 1962)

	Respiratory alkalosis in per cent	Respiratory acidosis in per cent	Oxygen desaturation in per cent
1. Asthmatic attacks	94		75
2. Status asthmaticus	66		72
		30	100
3. Long standing asthma without cardiac decompensation		48	100
	27		90
4. Long standing asthma with cardiac decompensation			
a. Temporary decompensation	32		100
b. Permanent decompensation		68	100

was lower than normal in 3 of the 4 patients. The CO_2-tension was lower than normal in 1 patient and higher than normal in 2.

During work the O_2-tension falls in 2 of the 4 patients. The CO_2-tension rises, however, during work in all of the patients.

According to HOLMGREN and LINDERHOLM (1958) the O_2-tension of arterial blood in healthy young people is lower during heavy work (pulse rate 168) than at rest, the mean difference being 17 mm Hg. The CO_2-tension, however tended to decrease during exhaustive work.

Therefore there exists a difference between asthmatic patients and healthy subjects what concerns the change in arterial CO_2-tension during work leading to an elevated CO_2-tension in patients with bronchial asthma during work. This fact may have importance for the production of tissue damage that can exist in severe asthma and which is evident from changes in the blood enzyme values (see under 4, Body tissues and COLLDAHL, 1943, 1960, 1961, 1964).

Thus 40 years ago with the help of spirometric and blood gas examinations Meakins could define the most important patho-physiologic phenomena in bronchial asthma: If the bronchial stenotic process is mild the result will be a moderate arterial hypoxemia caused by uneven venlation. If the stenoses are severe alveolar hypoventilation will develop with increased carbon dioxide tension and decreased oxygen tension.

2. *Blood electrolytes.*

Sodium is the cation of extracellular fluid and potassium the cation of intracellular fluid.

FENN (1939) has shown that partial occlusion of the arterial blood supply of a muscle results in an increase in the concentration of potassium in the venous plasma. From a stimulated muscle there is a loss of K, a gain of water and a gain of chloride.

Hyperkalemia has been found in different conditions and diseases such as asphyxia, anoxia, shock, asthma.

HARKINS (1941) considered that the increase of potassium in the blood was due to decrease of the blood volume and to cell lesions, that enabled potassium to leave them. Hyperkalemia also exists in anaphylactic shock both in dogs, rabbits and guinea pigs (SCHITTENHELM, ERHARDT, WARNAT, 1927; CORDA, 1934).

HALPERN, BRIOT, MONTON and TRUFFEST (1955) have shown that the injection of a histamine liberating substance causes hyperkalemia in the rat specially if the rat is adrenalectomized. In that case the potassium in the blood are more than doubled and all the animals die. The medication with an antihistamine preparation inhibits, more or less, the hyperkalemia produced by the histamine liberating substance. Therefore histamine has different effects: 1, a vascular effect; 2, a change in the permeability of the cells with diffusion of potassium from the cell.

MOJOVIC has found (1962) that in anaphylactic shock in guinea pigs the following occur:

1. Hyponatremia in the whole blood and blood plasma and slight hypernatremia in the erythrocytes.
2. Hyperkalemia in the whole blood and blood plasma followed by slight hypokalemia in erythrocytes.
3. Enhancement of blood cell volume in comparison with blood plasma volume.

TURIAF and collaborators, 1962, studied the electrolytes (Cl, Na, K) in spontaneous asthma. Only small changes were observed. A low Cl and Na and elevated K were occasionally found. (See table 3.)

TABLE 3

(Turiaf and collaborators 1962)

	Cl		Na		K	
Attack	101	(93-111) *	134	(108-148)	4,38	(3.8 -4.9)
Status asthmaticus	104	(98-114)	137	(126-143)	4,23	(2.85-5.1)
Long standing asthma:						
a. With decompensation	99	(90-104)	133	(130-139)	4.85	(4.1 -5.8)
b. Without decompensation	97.5	(86-109)	132	(122-148)	4.85	(4.1 -5.65)

* The values in brackets represents the lowest and highest value obtained.

Initials, sex, age, diagnosis	Date	Na mE/l	K mE/l	Ca mE/l	Cl mE/l	Total CO_2 mE/l
	1963					
G. L. male 03 04 13 Bronchial asthma	16.1					
	18.1					
	19.1					37
	20.1	133	4,9		96	23
	22.1	135	4,8		98	31,5
	23.1	133	4,5		97	34,5
	25.1	134	4,7		97	37,5
	28.1	144	5,0	4,2	97	39,5
	4.2	144	4,8		100	37,5
	20.2	139	4,3		104	38,0
	6.3	136	4,5		95	35,0
	20.3			5,2		28,0
	1960					
O. C. male 02 11 26 Bronchial asthma, emphysema pulm.	25.2					38,0
	27.2					32,5
	3.3					39,0
	10.3					
	11.3	137	7,4		87	37,5
	1961					
P .J. male 06 01 11 Asthma + Emphysema	30.8	120	4,5			
	2.9	150			102	
	21.9					
	28.9	145	4.5		96	41,5
	30.9	138	4,3		90	36
	3.10	142	5,8	4,5	109	
	5.10	152	4,9		107	

BLE 4

Standard Bicarbonate mE/l	P_{CO_2}	PH	Blood-pressure	GOT	GPT	ALD	LD	OCT	ICD
			150/90	71	61	10	30	11	
				62	37				
			170	2000	2000	25	420	50	
27	45	7,42	150	1980	1340	25	120	22	
24	43	7,39							
				300	290	23	70	15	
28				47	22	10	20	4	
			155/80	26	22	10	23	3	
				10	10	6	28	2	
				10	13	5	25	3	
			140/100	10	10	13			
				34	17	16	30		92
				20	10	10	2	9	81
				2400	1400	400	150	81	59
			105/60						
28,8			115/80	18	16	6	13	0	
30,5	63	7,42		20	26				
				20	26				
30	58	7,42	115/70	1100	950	150		150	
30,1				123	470				
25				42	185				

COLLDAHL has mostly found only small changes in Na, K and Cl even in cases with profound effect of the insufficient gas exchange on the organism as seen from the blood enzyme values. This is seen from table 4 which shows 3 patients with severe asthma. 2 of these patients died and had very profound changes in the tissues as a result from the insufficient gas exchange. One of these patients (O. C.) had, however, a very high K and a very low Cl value.

In a consecutive series of 42 asthma patients treated in S:t Göran's Hospital during 1964 determinations of Na, K, Ca, Cl, CO_2, were performed, table 5. It was found that both too low and too high values were obtained both for Na, K and Cl. 3-4 times as many low values were obtained as high ones. It is possible that the varying values can be explained in the same way as the oxygen uptake which can be either lower or higher than normal depending on which phase the asthma condition of the patient is in, e. g. low during very severe asthma, high in the period just following a period of severe troubles. However this may be it is quite certain that there is a disturbance in the electrolyte balance. The reason for this is most likely secondary to permeability changes of the cells caused by the allergic reaction or by processes connected with the insufficient gas exchange that exists in asthma.

From table 5 also it is quite clear that the patients in 2/3 of the cases had elevated blood values of carbon dioxide content. No patient had low values.

3. *Acid-base balance.*

From table 1, 2 and 6 it is clear that both respiratory acidosis and alkalosis can develop during asthma.

The changes are both respiratory and non respiratory (metabolic). The latter ones depend on the production of lactic acid caused by anaerobic metabolism. Asthmatic patients with asthma trouble have often slightly too high a value of lactic acid in the blood. (Investigated together with B. Pernow, table 6.)

As is seen from figure 1 the lactic acid production during work lies in the upper normal limit. The increase of the lactic acid content in the blood of asthmatic patients is with all certainty a sign of a tendency to anaerobic metabolism. For this interpretation the relatively low corresponding values for blood pyruvate speak (See table 6).

HUCKABEE, CARLSSON and PERNOW and others have investigated the oxygen utilization and lactate-pyruvate formation. They have found that the onset and degree of anaerobic work are best studied by simultaneous estimation of lactic and pyruvic acid concentrations in the blood.

An accumulation of blood lactate is observed not only in muscular work, but also in conditions where hypoxia is not present, for example after hyperventilation or administration of glucose or epinephrine. Consequently lactate alone is not a definite index of the adequacy of cellular oxygenation. In all the aforementioned conditions (exept hypoxia) in which lactate accumulates, a simultaneous increase takes place in py-

TABLE 5

mE/l	Number of determinations with Low values	Normal values	High values	Total	Number of patients With determinations	Without determinations	Total
Na	21	28	6	55	30	12	42
	126-136 *	137-148	149-156				
K	17	34	5	56	31	11	42
	2.5-3.7	3.8-5.1	5.2-9.4				
Ca	2	4	2	8	5	37	42
	3.9-4.0	4.1-5.1	5.2-5.3				
Cl	12	36	3	51	31	11	42
	84-95	96-107	108-110				
Co_2 (total content)	—	24	40	64	32	10	42
		22-30	31-32				

* The figures are the lowest and highest value in each group respectively.

TAE

(Colldahl, Ho

	Normal value	E. K., Male 20 09 20 Bronchial asthma	
		Rest	600 kp/min.
Sa_{O_2}	95-97 %		
Pa_{O_2}	85-95 mm. Hg.	88.5	88
Pa_{CO_2}	40 mm. Hg.	30	35
pH	7.38-7.47	7.52	7.45
Standard bicarbonate	21-26 m. Eq/l.	23	25
Pulse rate		82	149
Lactic acid mM/l.	$<$ 1.40 mM/l.	1.61	6.30
Pyruvic acid mM/l.	$<$ 0.15 mM/l.		

LE 6

ngren, Pernow)

	S. O., Male 25 09 13 Bronchial asthma		C. Y., Male 16 09 04 Bronchial asthma		H. L., Male 11 09 26 Bronchial asthma + Emphysema		
800 kp/min.	Rest	500 kp/min.	Rest	600 kp/min.	Rest	Work	Rest
					94	88	
90	76	60	69	75	68	58	84
36.5	39	54	44	50	46.6	58.4	50.8
7.38	7.45	7.29	7.43	7.33	7.46	7.37	
21	26	21.5	27	23	30		
171	83	137	80	139			
11.93	1.67	4.33	1.15	6.24	1.80		
	0.115	0.203	0.076	0.109			

ruvate (HUCKABEE, 1958). With an inadequate oxygen supply, reoxidation of the reduced diphosphopyridine nucleotide (DPNH) to its oxizidized form (DPN)—necessary for the carbohydrate breakdow to form high energy phosphate bonds—is channelled by reduction of pyruvate to lactate. Hypoxia, therefore, results in an increased formation of lactate from pyruvate (ASMUSSEN, 1950). As a measure of the degree of

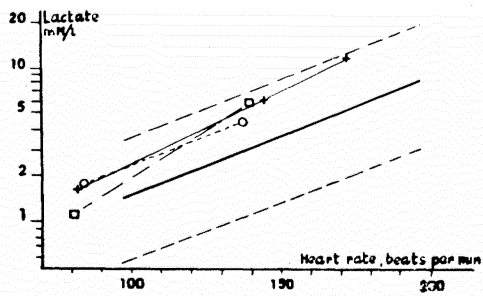

Fig. 1

Mean arterial lactate concentrations in relation to heart rate during exercise in healthy men between 30 - 39 years ——— . The 95 per cent confidence belt for single observations is marked - - - - - (according to T. STRANDELL, 1964).
The values for 3 asthmatic patients in this age group are marked x ——— x, o - - - - o and ◇ — . — ◇ (COLLDAHL, PERNOW).

oxygen deficiency, HUCKABEE (1958) used the calculation of "excess of lactate" (XL), i. e. the lactate change exceeding that which is directly proportional to pyruvate changes.

4. *Body tissues.*

It was shown by COLLDAHL (1943) that when guinea pigs are exposed to the simultaneous influence of oxygen deficiency and carbon dioxide in excess, some changes appear in the tissues that may be demonstrated as a lowered tissue respiration in various tissues both with the technique given by Warburg and with the methylene blue technique. It was possible to show that the altered tissue respiration resulted from a deficient break down of certain substances essential to the intermediate metabolism.

In 1960-64 it was shown (COLLDAHL) that tissue damage also exists in patients with severe asthma. This was shown by studying the pattern of serum-enzymes in asthmatic patients with varying degrees of asthma. A case was described (1960), who had a severe asthma and was in a very bad condition when he entered the hospital. He was trated in different ways, and he was also given artificial respiration in order to make his breathing more efficient. After some time he was better and the enzyme values became quite normal. One year later he had a new attack of asthma. His condition deteriorated and he subsequently died. At this time the enzyme values were again elevated. When this patient was examined post mortem no myocardial infarction could be found but

TABLE 7

Enzymes investigated	Number of asthma patients in per cent with a maximum enzyme value, one or more between: (The upper normal limit equals 100)						Total number of patients investigated
	0-100	100-200	200-300	300-500	500-1000	> 1000	
All (S - GOT, S - GPT, S-LD, S-MD, S-OCT, S-ALD, S-ICD)	24,4	43,6	19,8	8,7	2,3	1,2	172
S-GOT	57	30,8	7,0	2,8	1,2	1,2	172
S-GPT	68,4	18,8	7,0	3,4	1,2	1,2	171
S-LD	88,9	9,9	0,6	—	—	0,6	171
S-MD	88,1	10,2	—	1,7	—	—	59
S-OCT	58,5	28,7	9,8	1,8	0,6	0.6	164
S-ALD	67,5	19,3	9,0	3,6	0.6	—	166
S-ICD	94,6	2,7	2,7	—	—	—	74

TABLE 8

Normal ranges of serum enzyme activities por ml.

Enzyme	Range	
	Regular units	Relative units
Aldolase (ALD)	3-8	37-100
Glutamic oxalacetic transaminase (GOT)	8-30	27-100
Glutamic pyruvic transaminase (GPT)	5-35	14-100
Lactic dehydrogenase (LD)	140-290	48-100
Malic dehydrogenase (MD)	30-80	37-100
Ornithine carbamyl transferase (OCT)	0-6	0-100
Isocitric dehydrogenase (ICD)	< 265	< 100

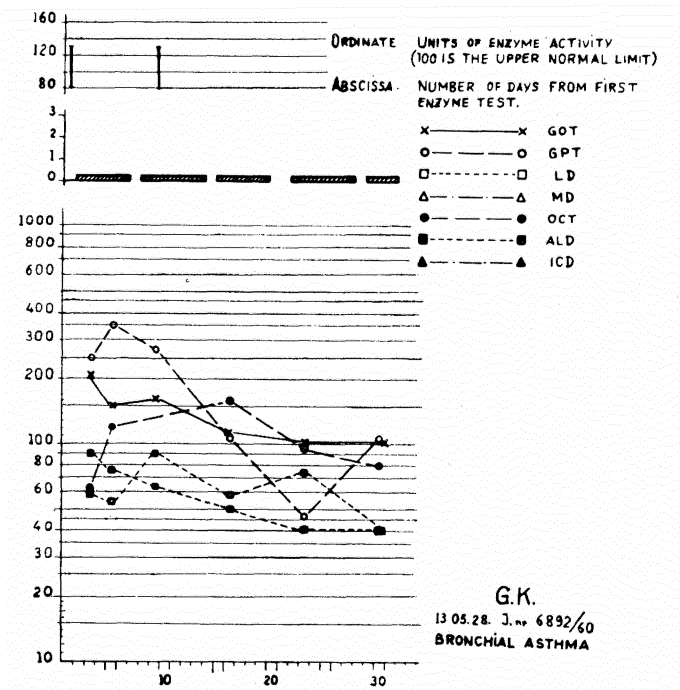

Fig. 2

Ordinate: 1. Blood pressure.—2. The severity of asthma (1 = mild, 2 = medium, 3 = severe).—3. Units of enzyme activity (100 is the upper normal limit).
Abscissa: Days after admission in hospital (26.9 1960).
Diagnos.: Asthma bronchiale + Tbc pulm sanata.
G. K. Born 13 05 23.
Asthma bronchiale since 1925. Since childhood also nose and eye troubles. Slight sensitivity to dust and animal dander. Shortness of breath especially when working.
ECG normal (11th day after admission).
X-rays (lungs and heart) normal. (September 1960.)
BSR 3 mm/hour 2nd day after admission.
7 mm/hour 8th day after admission.
29 mm/hour 14th day after admission.
10 mm/hour 21st day after admission.

the were changes in the liver (centrilobular necrosis). These liver changes are with all certainty caused by insufficient gas exchange.

The methods used for the enzyme determinations are discussed in earlier papers (1960-1964). The results from determination of different

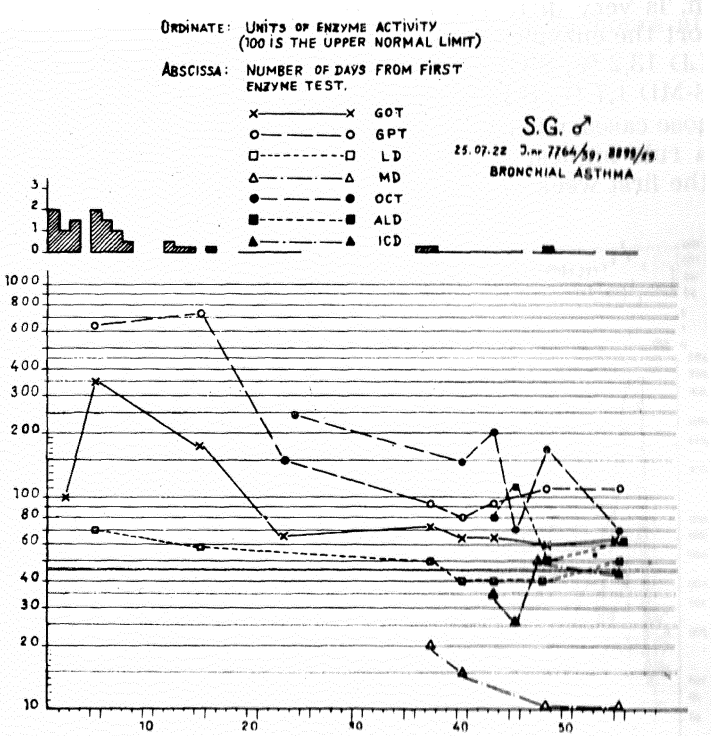

Fig. 3

Ordinate: 1. The severity of asthma (1 = mild, 2 = medium, and 3 = severe). 2. Units of enzyme activity (100 is the upper normal limit).

Abscissa: Dates from the 23rd October 1959.

Patient S. I. G. Born 25.7 1922. Journals No. 7764/59 and 8899/59. The patient had asthma for the first time in January 1950 after a respiratory infection. Since then he has had asthma particularly in Autumn. In the middle of October 1959 he had further attacks of asthma. An X-ray of the sinuses of the nose on the 13th day after admission was normal. An X-ray of the lungs on the 43rd day after admission revealed slight emphysema. ECG on the 9th day was normal. Skin Test negative.

Sedimentation rate 9 mm/hour (2nd day).
5 mm/hour (19th day).

Eosinophils reached a maximum of 23 %. White blood Corpuscle Count varied between 7 800 and 15 400.

ACTH was first given on the 29th October. No steroids were given. The enzyme pattern changes are seen from Fig. 3.

blood enzymes can be seen in table 7. The normal ranges of serum enzymes activities per ml are found in table 8. In the figures relative units have been used, where the upper normal limit for the different enzymes was put at 100, since the units of enzyme activity differ considerably numerically (table 8).

From table 7 we can see that 32 % of the patients had at least

one enzyme value over 200 %. 12,2 % had at least one value more than 300 %.

The number of patients, who had at least one enzyme value more than 200 % of the normal value where one can reckon with a significant elevation, is very different for the different enzymes investigated. In this report the enzymes come as follows:

S-ALD 13,2 %, S-GPT 12,8 %, S-OCT 12,8 %, S-GOT 12,2 %, S-ICD 2,7 %, S-MD 1,7 %, S-LD 1,2 %.

In those cases, where the patients have elevated enzyme values these are as a rule highest when the patients are admitted to hospital and during the first week of the hospital stay. When the patients get better

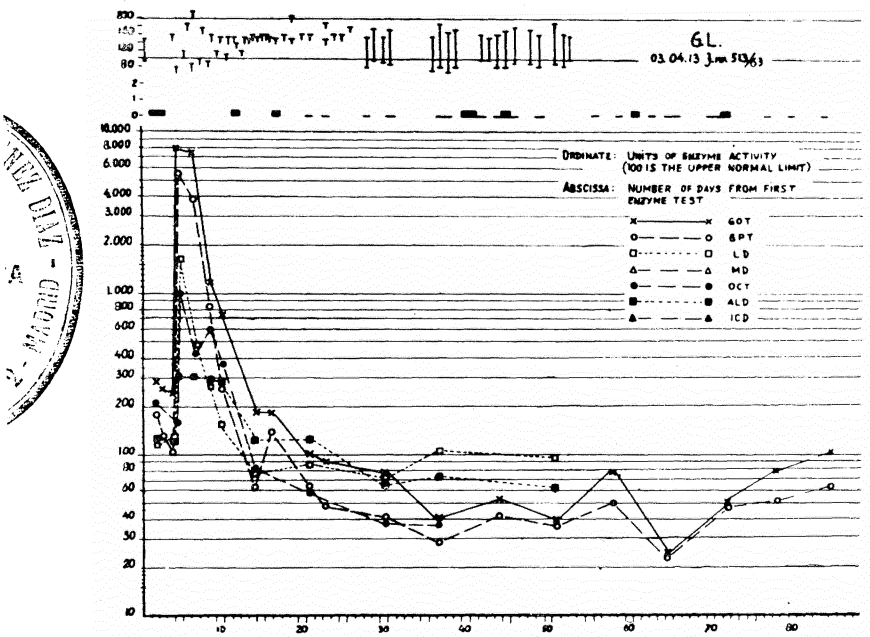

Fig. 4

Ordinate: 1. Blood pressure.—2. The severity of asthma (1 = mild, 2 = medium, 3 = severe).—3. Units of enzyme activity (100 is the upper normal limit).
Abscissa: Days after admission in hospital. (15.1 1963).
Diagnos.: Asthma bronchiale + Bronchopneumonia.
G. L. Born 03 04 13.
Asthma since 1933 periodically. 2 weeks before admission (January 15, 1963) more asthmatic troubles. Last week not possible to perform any work.
Some days after admission the patient became somnolent and cyanotic. Treated with artificial respiration from the 4th day for about 2 weeks.
ECG (2nd day after admission): almost normal, tachycardia 100/min.
(6th day after admission): almost normal, tachycardia 135/min.
No signs of myocardial infarction.
(9th day after admission): almost normal, tachycardia 110/min.
(11th day after admission): almost normal, tachycardia 85/min.
(17th day after admission): almost normal, tachycardia 90/min.
Skin Test negative (13.3 1963).
X-ray thorax (4th day) Small lung infiltrations right side. Heart normal.
BSR 93 mm/hour (2nd day).
24 mm/hour (36th day).

the enzyme values go down. If the patient subsequently has more severe asthmatic troubles the values may rise again.

In asthmatic patients it is not only the degree of breathing difficulty but also the duration of respiratory insufficiency that is of importance for the enzyme pattern. One of our patients, who had a sudden and very severe attack of asthma and died within the first 24 hours after the

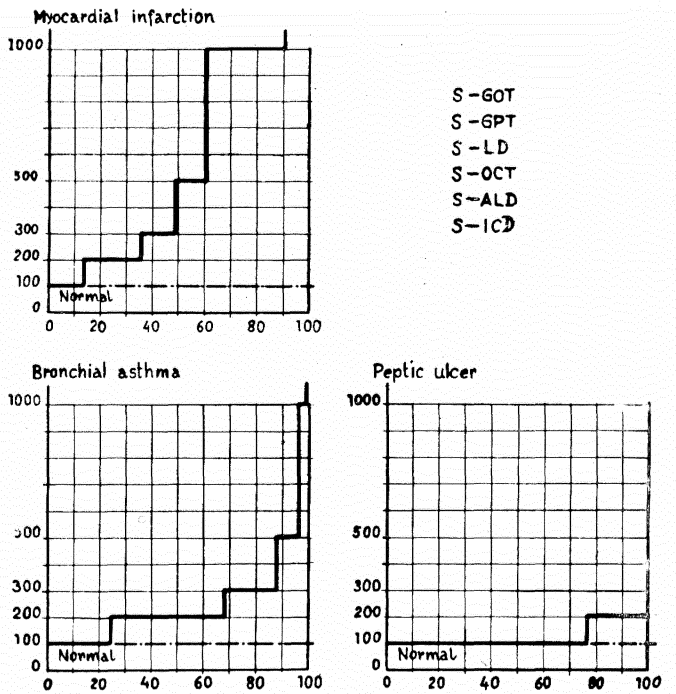

Fig. 5

The frequency of elevated blood enzyme values (S-GOT, S-GPT, S-LD, S-OCT, S-ALD, S-ICD) in three different series of patients with asthma bronchiale, myocardial infarction and peptic ulcer. For each patient the highest value of any of the blood enzymes investigated determined the group to which the patients should belong.
Ordinate: Enzyme value. 100 is the upper normal limit.
Abscissa: Asthma bronchiale: 100 = 172 patients. Myocardial infarction: 100 = 51 patients. Peptic ulcer: 100 = 30 patients.

breathing trouble started, had no elevated enzyme values. Further patients with severe enphysema need only slight aggravation of breathing difficulty in order to obtain a pathological enzyme pattern.

Fig. 2-4 show different types of enzyme patterns in asthmatic patients with from slight to severe asthma.

Fig. 5 shows graphically the frequency of elevated enzyme values (all investigated) in bronchial asthma, myocardial infarction and peptic ulcer.

Therefore tissue damage occurs in connection with severe asthmatic troubles of longer duration, just as in experimental asthma in guinea

pigs. A definite elevation of S-OCT proves the existence of liver damage.

It is of interest, that, according to the only earlier report of enzyme determination in asthma I know (STEINMANN, WIDMER, KAMMER) the diastase, the only enzyme investigated by the authors mentioned, was elevated in asthmatic attacks. I have never found any elevation of the diastase even if the patient had strongly elevated values of S-GOT, S-GPT, S-LD, S-OCT and S-ALD.

The serum values of such enzymes as S-ALD, S-GPT, S-OCT, S-GPT are therefore not infrequently elevated in patients with severe asthma, especially in cases of long duration. Generally this indicates tissue damage, that can be caused by the insufficient gas exchange.

An elevated GOT value in a patient with severe asthma does not primarily indicate that there is simultaneous coronary obstruction, but usually indicates tissue damage that can be caused by insufficient gas exchange.

Strongly elevated enzyme values in patients with insufficient gas exchange often indicate that the patients are in a bad condition and may need artificial respiration in order to have the pulmonary gas exchange normalized.

SUMMARY.

In bronchial asthma the tensions of oxygen and carbon dioxide in the blood are often changed.

The disturbances of the blood gases can provoke changes in blood electrolytes, acid base balance and the different tissues of the body, especially the liver. These changes can be so pronounced that they are incompatible with the continuance of life. The disturbances therefore are to be normalized as soon as possible by therapeutic means.

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After this paper was sent in for publication the following work concerning "Serum Transaminase Activity in Status Asthmaticus" has been published in *Brit. Med. J.*, 9 May 1964, vol. 1, 1220-1223, by A. H. EL-SHABOURI, A. J. THOMAS, D. A. WILLIAMS.

AUSWIRKUNGEN DES CHRONISCHEN BRONCHIALASTHMAS AUF HERZ UND KREISLAUF

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Das *Asthma bronchiale* definieren wir im deutschen Sprachgebiet als Krankheitsbild, bei welchem es infolge funktioneller Obstruktionen im Bronchialsystem zur Stenoseatmung kommt. Diese Behinderung der Atmung wird subjektiv als Dyspnoe empfunden. Objektiv imponieren vor allem die Verlängerung des Exspiriums und die trockenen Rasselgeräusche wie Giemen, Pfeifen und Brummen. Auf die verschiedenen ursächlichen Faktoren kann hier nicht eingegangen werden.

Das Asthma bronchiale tritt in seiner reinsten Form als *akutes Anfallsleiden* mit beschwerdefreien Intervallen auf. Nach Jahre-bis Jahrzehnte-langer Dauer der Anfallsrezidive führt die begleitende zeitweise Longenblähung dann unter fortschreitender Schwächung des elastischen Lungengerüstes zu anatomischen Alterationen der Lungen, die schliesslich fixiert werden und irreversibel sind. Der Endzustand ist das generalisierte, chronisch substantielle Lungenemphysem. In diesem Stadium spielen ausser fuktionellen Momenten morphologische Störungen eine vorherrschende Rolle.

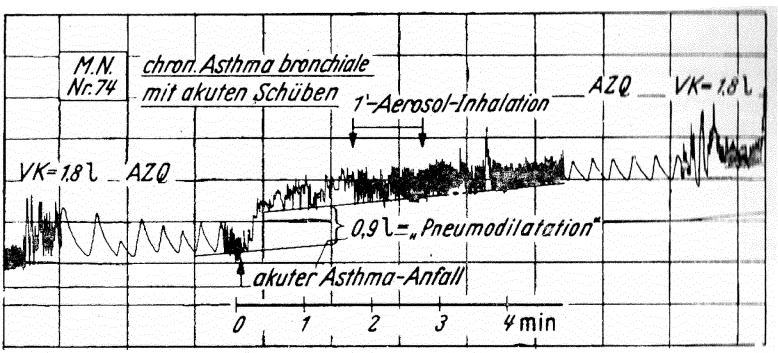
Neben diesem akuten Asthma bronchiale, welches zunächst intermittierend verläuft, existiert die *chronische Asthmakrankheit.* Auch sie ist charakterisiert durch Stenoseatmung und exspiratorische Dyspnoe, aber oft ohne Anfallscharakter, dafür mit ständig vorhandenen, wechselnd intensiven Beschwerden. Die Übergänge zur asthmoiden Bronchitis, welche von Anfang an chronisch verläuft, sind flissend. Beide führen schliesslich ebenfalls zum generalisierten, chronisch substantiellen Lungenemphysem.

Bei der *Diskussion über die Auswirkungen des chronischen Bronchialasthmas auf Herz und Kreislauf* stehen somit zunächst ein Dehnungsemphysem und dann später *das generalisierte, chronisch substantielle Lungenemphysem* im Vordergrund. In diesem Stadium finden wir eine erhebliche Einschränkung des Lungencapillarbettes und eine respiratorisch bedingte Mangelbelüftung der Lungenalveolen als charakteristische Folgen. Sie bewirken eine *pulmonale Hypertonie.* Auf Grund dieser chronisch erhörnten Druckbelastung entwickelt sich schliesslich *ein chronisches Cor pulmonale.*

Einige Abbildungen aus unserem Arbeitskreis um KNIPPING mögen diesen Ablauf verdeutlichen.

Abbildung 1 zeigt den Beginn eines akuten asthmatischen Anfalles im Spirogramm. Charakteristisch sind die plötzliche Verschiebung der Atemmittellage um 900 ccm zur inspiratorischen Seite hin und die schwere Dyspnoe in Form der undulierenden Ventilation. Nach Bronchovydrin-Gaben bilden sich die Zeichen schnell zurück.

Abbildung 2 demonstriert die Auswirkungen von Valsalva-Versuchen gegen 30 bzw. 60 mm Hg auf die Lungencapillaren. Diese Pressdruckübungen können als extreme Modelle eines Volumen pulmonum auktum



Ab. 1

gewertet werden, wenn auch andere Mechanismen noch eine wesentliche Rolle spielen. Wie durch die gleichzeitige Herzkatheterung nachweisbar ist, kommt es zu entsprechend grossen Drucksteigerungen in den Pulmonalcapillaren.

Die Abbildungen 3 und 4 geben Aufnahmen von selektiven Lungenangiogrammen bei einer Normalperson wieder. Charakteristisch sind zunächts die zahlreichen grosskalibrigen mittleren Lungenarterien mit astförmigen Verzweigungen. In der nächsten Phase wirkt die Wipfelfülle des peripheren pulmonalen Gefässbettes eindrucksvoll. Es ist das normale Bild der intakten und voll funktionsfähigen Lungencapillaren.

Die Abbildungen 5, 6 und 7 demonstrieren das Verhalten der Lungengefässzeichnung bei hochgradigem Lungenemphysem. Auffällig sind die erhebliche Ektasie der zentralen Pulmonalarterie, die Verschmälerung des Kalibers und das besenreiserartige Muster der mittleren Pulmonalarterien sowie das weitgehende Fehlen des Capillarbettes. Letzeres kommt als Wipfeldürre der peripheren Lungengefässe zur Darstellung.

Das chronische Cor pulmonale als Auswirkung eines chronischen Bronchialasthmas entwickelt sich im allgemeinen ausgesprochen langsam und spät. Es ist das Endergebnis eines Jahre- bis Jahrzehnte-langen Krankheitsablaufs und wird deshalb meist erst im Alter von 45 bis 65 Jahren beobachtet. Wir verstehen unter dem chronischen Cor pulmonale die Veränderungen am rechten Herzen, welche als Folge einer langdauernden Mehrarbeit des Herzmuskels auf Grund der erhöhten Widerstände im kleinen Kreislauf auftreten. Es ist zuerst die Hypertrophie

des rechten Ventrikels, dann seine Dilatation und schliesslich die Insuffizienz.

Subjektiv beginnt der Patient im Verlauf dieses Geschehens zusätzlich über Herzsensationen zu klagen. *Objektiv* findet sich dann häufiger eine Sinustachykardie. Man hüte sich aber davor, jede Tachykardie bei

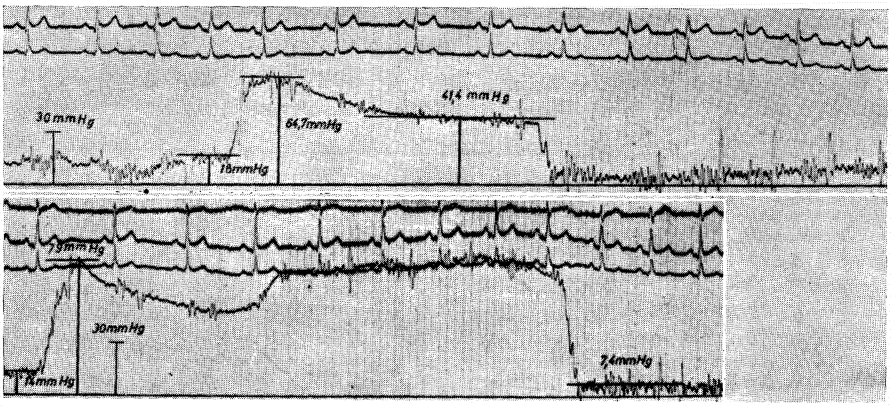


Abb. 2.—Druckveränderungen in den "Lungenkapillaren" unter den Bedingungen des Pressdrucks. Die in Abb. 1-7 mit A gekennzeichneten Kurven wurden bei einem Pressdruck von 30 mm Hg, die mit B bezeichneten Kurven bei einem Pressdruck von 60 mm. Hg geschrieben. ("PC"-Druck nach der Pressdruckprobe in Abb. 1 B: 7,4 mm Hg.)

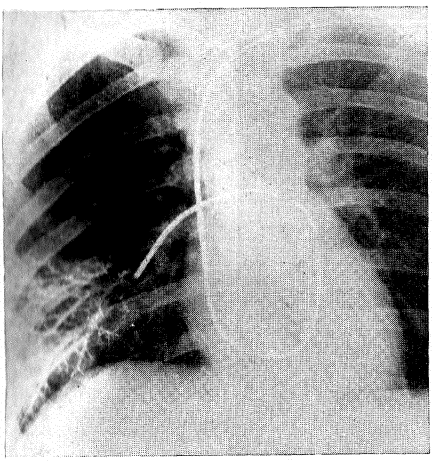
den ohnehin vegetativ-labilen Asthmatikern als Ausdruck einer organischen Herzläsion zu bewerten. Ein niedriger Blutdruck, ein stärkerer rechter Ventrikel mit sichtbaren oder tastbaren epigastrischen Pulsationen, ein verstärkter 2. Pulmonalton und oft leise Herztöne sind weitere Hinweise.

Röntgenologisch kann die Beurteilung der Herzsilhouette zu falschen Schlüssen führen. Hinter dem häufig ausgesprochen schmal und langgestreckt erscheinenden Herz-Gefäss-Band kann sich eine beträchtliche Hypertrophie der rechten Kammermuskulatur verbergen. Emphysem und Zwerchfelltiefstand tragen dazu bei, dass die effektiv vorhandene Vergrösserung des Herzens nicht in einer Verbreiterung seines Schattens zum Ausdruck kommt. Ein vorspringender Pulmonalisbogen und verstärkte Hiluspulsationen sind in diesem Zusammenhang immer bemerkenswert.

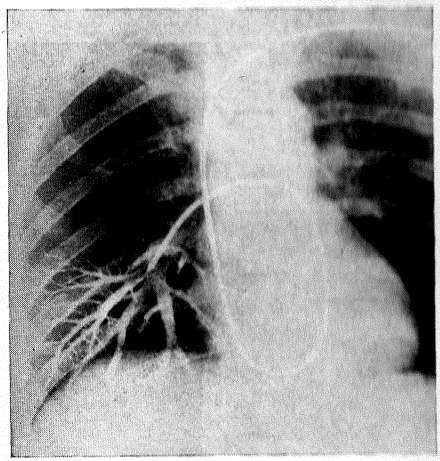
Die EKG-Untersuchung ist für die Diagnostik des chronischen Cor pulmonale nicht so ergiebig, wie man gemeinhin annehmen sollte. Die Zeichen und Befunde sind, wenn vorhanden, zunächst nicht für das Cor pulmonale, sondern für die *rechtsventrikuläre Hypertrophie* charakteristisch. Dabei sei daran erinnert, dass letzture im EKG deutlich erkennbar wird, wenn das Verhältnis zwischen den Muskelmassen der rechten und linken Kammer etwa 1:1 beträgt oder zu Gunsten der rechten Kammer überwiegt. Nach DONZELOT und Mitarbeiter, sowie SCHMIDT unterscheidet man auf Grund von Studien über Hämodynamik und EKG

verschiedene Ursachen und Formen sowie nach HOLZMANN drei Grundtypen im EKG.

Die Abbildung 8 bringt eine schematische Darstellung der QR-Zeit im normalen Brustwand-EKG (a) und bei Rechtshypertrophie (b). Die

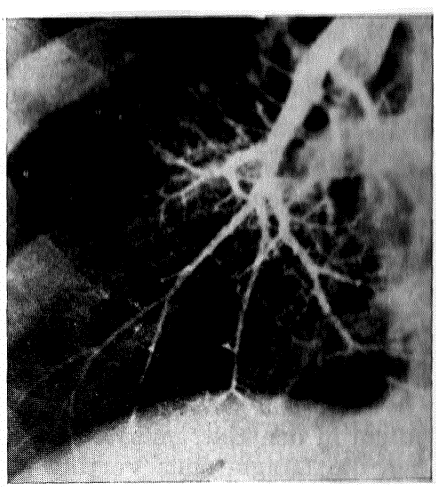


Ab. 3



Ab. 4

Ab. 5



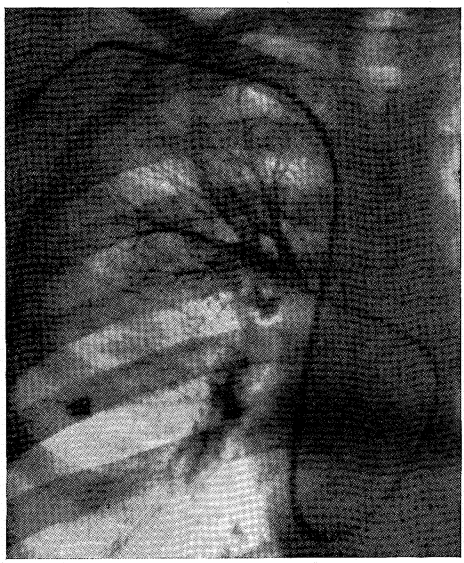
Ab. 6

Kennzeichen einer extremen Widerstandshypertrophie finden sich bei c.

In der speziellen Situation des chronischen Bronchialasthmas und des ausgeprägten Lungenemphysems handelt es sich um eine Widerstandshypertrophie mit vermehrter Druckarbeit der rechten Kammer ohne Entlastungsmöglichkeit sowie um eine rechtsventrikuläre Hypertrophie mit Vektorprojektion nach rechts hinten. Neben der reinen Hy-

pertrophie sowie meist sekundär auch Dilatation der rechten Kammer sind eine Steilstellung des Herzens, eine Rückwärtsverlagerung der Herzspitze und eine Drehung um die Längsachse im Gegensinne der Uhrzeigerbewegung zu nennen.

In diesem Zusammenhang sei daran erinnert, dass ganz allgemein



Ab. 7

die wichtigsten elektrokardiographischen Zeichen einer durch Mehrarbeit verursachten Herzhypertrophie folgende sind:

1. Die Grössenzunahme der Ausschläge der Erregungsausbreitung und die Verbreiterung der Zone grosser R-Zacken.
2. Die Drehung des Summationsvektors von QRS in der Frontal und Horizontalebene.
3. Die Verlängerung der QR-Dauer.
4. Die Änderung der Erregungsrückbildung.

Lage-und Dreh-Bewegungungen des Herzens kommen im EKG vor allem in den Extremitäten-Ableitungen zum Ausdruck. Eeine Drehung um die Längs- oder Vertikal-Achse verändert den EKG-Typ wechselsinnig, d. h. eine Drehung nach links ruft einen Rechtstyp hervor und umgekehrt. Drehungen um die transversale Achse lassen oft unbestimmbare oder überdrehte Typen erscheinen, wobei an der vorderen Brustwand die S-Zacken überwiegen, wenn die Herzspitze mehr hinten liegt.

Folgende Zeichen dürfen in Praxis und Klinik bei der üblichen EKG-Einzeluntersuchung als *Kriterium einer rechtsventrikulären Hypertrophie* gelten:

1. *Eine grosse schlanke R-Zacke in den rechtspräkordialen Ableitungen.* Nur wenn die R-Zacke oder R'-Zacke grösser als die S-Zacke

ist, selbst mindestens 1/2 mV misst und nicht gleichzeitig ein Schenkelblock besteht, dann spricht das Kurvenbild unmittelbar für eine Rechtshypertrophie. Diese R-Zacke soll jedenfalls grösser als die in der Ableitungsreihe weiter links gelegenen R-Zacken sein (Ableitung V_3, V_4).

2. Bezeichnend für eine Rechtshypertrophie ist in den präkordialen Ableitungen eine *Zunahme im R/S-Verhältnis von links nach rechts.*

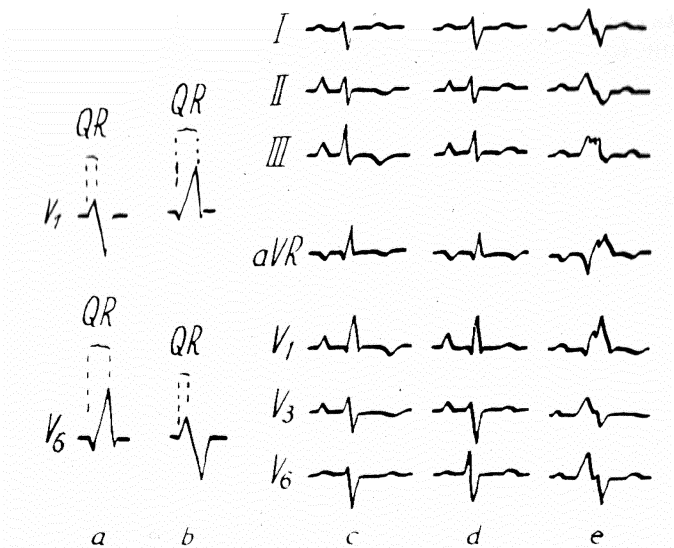


Abb. 8.—Schematische Darstellung der QR-Zeit im normalen Brustwand-Ekg (*a*) und bei Rechtshypertrophie (*b*). Kennzeichen der "extremen" Widerstandshypertrophie in *c*, der Anpassungshypertrophie in *d*, der Volumenhypertrophie in *e*.

Nach links nehmen die R-Zacken an Grösse ab, die S-Zacken dagegen zu. Es handelt sich um das sogenannte Wilson-Phänomen.

3. Bei einer vertikalen Herzachse gilt auch *eine grosse R-Zacke in der Ableitung aVR* als Zeichen einer Rechtshypertrophie.

4. Wenn keine Leitungsstörung besteht und die QR-Zeit bestimmbar ist dann wird man eine Rechtshypertrophie annehmen müssen, solbald *die Differenz von QR in V_6 minus QR in V_1 aufgehoben oder negativ* wird.

Es sind noch zahlreiche weitere EKG-Zeichen und -Relationen beschrieben worden, die sich aber fast sämtlich auf diese Grundkriterien einer Rechtshypertrophie zurückführen lassen. Rechtsventrikuläre und Sternal-Ableitungen können weitere Aufschlüsse bringen.

Für das chronische Cor pulmonale sind noch folgende *Zeichen bei Überlastung des rechten Vorhofs und bei Leitungsstörungen* von Interesse:

1. *Abweichung der elektrischen Achse nach rechts und Ausbildung eines Rechtstyps.* Diese soll nur zur Diagnose der Rechtshypertrophie herangezogen werden, wenn gleichzeitig andere EKG-Zeichen für eine solche sprechen.

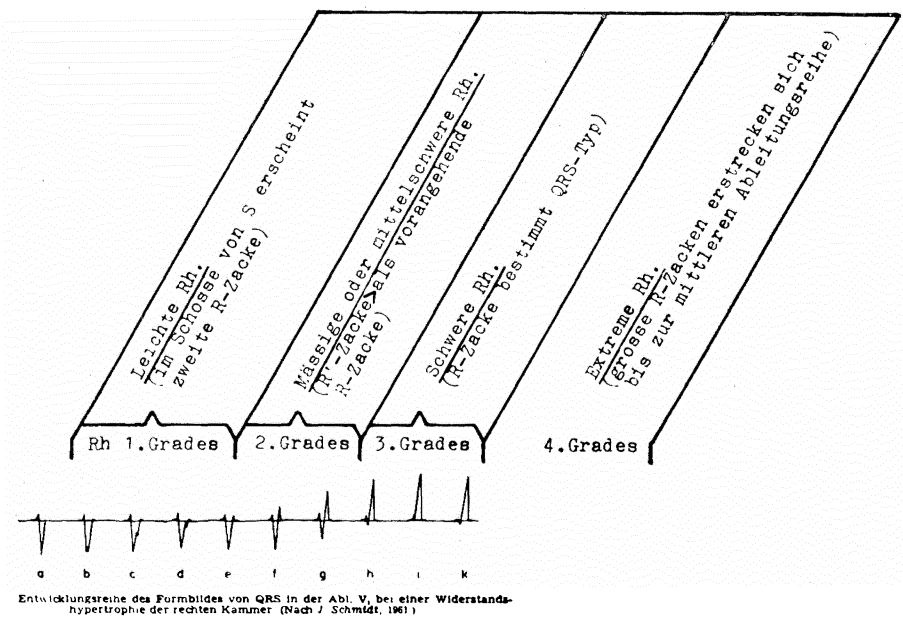
2. *Partielle oder totale Rechtsschenkelblockbilder* können auf-

treten Es besthet jedoch keine Abhängigkeit zur Schwere der pulmonalen Hypertonie oder der Hypertrophie.

3. *Negative T-Zacken rechts präkordial.*

4. *Veränderungen der P-Zacken, und zwar prominente spitze Vorhofzacken in den Extremitäten-Ableitungen II und III und in aVF.* Weiter hohe, meist diphasische P-Zacken rechts präkordial mit zuerst hohem, spitz positivem, dann kurzem negativen Anteil.

Auch eine *Einteilung der Rechtshypertrophie im EKG nach Schwe-*



Ab. 9.

regraden ist erfolgt. Hierbei ist für den Entwicklungsgrad der Rechtshypertrophie der Stand des Umbaues von QRS in den präkordialen Ableitungen ein klinisch brauchbares Mass.

Die Abbildung 9 demonstriert ein Schema.

Eine Rechtshypertrophie ersten oder leichten Grades liegt dann vor, wenn im Schosse von S rechts präkordial eine zweite R-Zacke erscheint. Von einer Rechtshypertrophie zweiten, mässigen oder mittelschweren Grades spricht man, wenn die R'-Zacke in Ableitung V_1 grösser als die vorausgehende R-Zacke geworden ist, wenn links präkordial S-Zacken erscheinen und die Übergangszone nach links verlagert ist. Eine Rechtshypertrophie dritten oder schweren Grades wird dann diagnostiziert, wenn annähernd eine Umkehr des normalen Formbildes erreicht ist und die R-Zacke in Ableitung V_1 oder V_3R den QRS-Typ bestimmt. Es liegt dann eine Rechtshypertrophie von der Stärke der linken Kammer vor. Eine Rechtshypertrophie vierten oder extremen Grades wird dann angenommen, wenn das Formbild grosser R-Zacken,

gefolgt von negativen T-Zacken, sich von rechts präkordial bis zur mittleren Ableitungsreihe erstreckt. Diese Gradeinteilung gibt auch nach unseren Erfahrungen eine recht gute Auskunft über den Schweregrad der Rechtshypertrophie.

Die Bedeutung des chronischen Cor pulmonale wurde bei 126 Patienten mit einem chronischen Bronchialasthma bzw. nachfolgenden Lungenemphysem überprüft. Die weitaus überwiegende Zahl der Probanden hatte ein Alter zwischen 30 und 75 Jahren erreicht. Berücksichtigung fanden nur Patienten mit einer mehr als 5 Jahre laufenden eindeutigen Anamnese. In Einzelfällen betrug sie mehr als 25 Jahre. Die oben aufgeführten Kriterien der rechtsventrikulären Hypertrophie im EKG standen bei dieser Analyse zur Diskussion. Einzelne oder mehrere Hinweise für ein chronisches Cor pulmonale fanden wir in insgesamt 27% der Fälle. Bei weiteren 9% war das Ergebnis der EKG-Exploration fraglich. Die zusätzliche Untersuchung mit rechtspräkordialen und Sternal-Ableitungen in den negativen Fällen konnte bei einer weiteren kleinen Gruppe zusätzliche Hinweise für eine Rechtshypertrophie liefern.

In den ersten 10 Jahren eines anfallsartigen Bronchialasthma waren die EKG-Zeichen äusserst selten. Erst mit längerer Laufzeit und allmählicher Entwicklung eines ausgeprägten Lungenemphysems nahm das Cor pumonale an Häufigkeit zu. Die Dauer des Krankheitsgeschehens hat somit eine erhebliche Bedeutung. Aber auch das Lebensalter und die noch gerforderten Arbeitsleistungen im beruflichen Alltag scheinen einen Einfluss auszuüben. Mehrere ältere, seit Jahren weitgehend inaktive Patienten zeigten trotz langer Bronchialasthma-Anamnese keine Hinweise für ein Cor pulmonale im EKG. Bei der kritischen Analyse der Gesamtsituation müssen selbstverständlich auch die Verhältnisse des linken Ventrikels und die eventuell zusätzlichen Druckbelastungen durch eine Altershypertonie berücksichtigt werden.

Die Aufschlüsselung der EKG-Hinweise für ein cor pulmonale nach dem jeweiligen Schweregrad zeigte, dass bei einem chronischen Bronchialasthma weitaus überwiegend Rechtshypertrophien ersten oder leichten Grades, bzw. zweiten, mässigen oder mittelschweren Grades verursacht wurden. Eine Rechtshypertrophie dritten oder schweren Grades kam nur in ganz seltenen Einzelfällen zur Beobachtung.

Abschliessend zeigen die hier diskutierten Auswirkungen des chronischen Bronchialasthmas auf Herz und Kreislauf wiederum die für Praxis und Klinik so bedeutsamen engen Beziehungen zwischen Atmung und Zirkulation auf.

BERUFSBEDINGTE SENSIBILISIERUNG BEIM BRONCHIAL-ASTHMA

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Unter den exogen-allergisch bedingten Asthmaformen und ihren Äquivalenten nimmt das berufsbedingte allergische Bronchialasthma in vielfacher Beziehung eine Sonderstellung ein. Diese Unterschiede sind zugleich Anlass und Berechtingung, das professionelle allergische Bronchialasthma einer gesonderten Betrachtung zu unterziehen. Dabei halte ich es in diesem *Übersichtsreferat* für meine Aufgabe, einen zusammenfassenden Bericht unserer heutingen Kenntnisse zu geben, der sich auf das Grundsätzliche beschränkt. Ich muss daher notgedrungen darauf verzichten, einen Überblick über die umfangreiche Literatur und die vielfältige, z. T. äusserst wichtige und interessante Kasuistik zu geben. Eine solche Darstellung wäre vielmehr das Anliegen einer bibliographischen Bearbeitung meines Themas. Ich bitte daher um Ihr Verständnis, wenn ich mich auf wesentliche Gesichtspunkte der *Pathogenese* und *Ätiologie,* sowie einiger *diagnostischer* und *soziologischer* Probleme beschränke.

Wenngleich die allergische Pathogenese nur *eine* der *möglichen* Entstehungsweisen des professionellen Asthma darstellt, so ist die Vermutung berechtigt, dass diese thematische Beschränkung bewusst getroffen werden musste. Der Grund hierfür dürfte in erster Linie dadurch gegeben sein, dass das allergische professionelle Asthma im Gegensatz zu dem primär chemisch-irritativen (toxischen), wie besonders aber zu dem physikalisch-irritativen (durch Wärme, Kälte und durch inerte Stäube) bedingten Asthmaformen ein abgerundetes klinisches Bild, eine zuverlässige und zumutbare Diagnostik, kausale therapeutische Möglichkeiten wie auch gesicherte Grundlagen für eine sozialrechtliche Beurteilung (Entschädigungspflicht) besitzt. Ich kann daher die den *nicht* allergischen professionellen Asthmaformen heute noch anhaftende vielfältige Problematik hier übergehen.

Im Gegensatz zu dem "spontanen" allergischen Bronchialasthma durch Sensibilisierung gegen Antigene unseres "natürlichen" Lebensraumes ist das professionelle allergische Bronchialasthma durch besondere berufsspezifische Stigmata geprägt. Denn unter dem Aspekt einer möglichen Sensibilisierung unterscheidet sich das Berufsmilieu im wesentlichen in zweierlei Hinsicht von unserem natürlichen Lebensraum:

1. Durch die spezielle berufsbedingte Exposition.

2. Durch die antigene Potenz und Aggressivität der verschiedenen Arbeitsstoffe.

Die Exposition gegenüber dem Arbeitsstoff ist in erster Linie durch die *zeitliche* Dauer ausgezeichnet, d.h. durch die meist monate- und jahrelang sich ständig wiederholende Begegnung mit dem Antigen. Im Gegensatz zu vielen spontanen Sensibilisierungen durch irgendeln Gelegenheitsantigen gesteht beim professionellen allergischen Bronchialasthma eine *kontinuierliche* Exposition. Den *konstanten* Expositionsbedingungen, die das Arbeitsmilieu mit sich bringt, ist der Werktätige mit seiner *variablen* stets schwankenden *individuellen Disposition* ausgesetzt. Diese ist—wenn auch von Fall zu Fall in Bezug auf die aktuelle "biologische Stimmung" wechselnd und im Stellenwert ihrer einzelnen Faktoren (Infekt, interkurrente Erkrankungen, psychische Verfassung, etc.) kaum abgrenzbar—entscheidend für die Realisierung des Sensibilisierungstermines, bzw. für die unterschiedliche Dauer der Expositionszeiten bis zum Auftreten manifester Erscheinungen.

Ausser der *Kontinuität* des Antigeneinstromes in Verbindung mit der wechselnden *idiodispositionellen* Sensibilisierungsbereitschaft ist die professionelle Exposition gegenüber gewerblichen Antigenen weiterhin und wesentlich gekennzeichnet durch ihre *besondere Intensität*. Sie ist bedingt durch den technischen *Arbeitsvorgang*, durch die speziellen Verhältnisse des *Arbeitsplatzes* und schliesslich durch den Wechsel und Wandel der *Arbeitsmethodik*. Die Erhebung der Anamnese hat daher besonders zu achten auf die genaue Erfassung des *Arbeitsvorganges*. Sie darf sich keinesfalls mit allgemeinen Berufsbezeichnungen wie etwa "Weber" begnügen. Denn hieraus ist nicht ersichtlich, welche Arbeitsstoffe verarbeitet werden, ob etwa Rohseide oder entbastete Seide, ob Kunstseide, ob gefärbte oder ungefärbte Seide, ob Wolle oder Baumwolle, ob Kunststoffe u. a. mehr. Die Anamnese muss fernerhin die speziellen Verhältnisse am jeweiligen *Arbeitsplatz*, wie Klimatisierung, Belüftung, Staubentwicklung etc. berücksichtigen. Von grosser Wichtigkeit sind z.B. die in dem gleichen Industriezweig von Betrieb zu Betrieb wechselnden Einrichtungen des Arbeitsschutzes, wie etwa das Vorhandensein von Absaugvorrichtungen und anderen technischen Einrichtungen. Ein bekanntes Beispiel ist das Bäckerasthma, das in Kleinbetrieben wegen der mangelnden technischen Ausrüstung weitaus häufiger in ca. 44%, in modernen Grossbetrieben hingegen nur in ca. 25% auftritt. Trotz gründlicher Anamneseerhebung wird in vielen Fällen in Anbetracht des ständigen Wandels der Arbeits-und Fabrikations-methoden sowie der Entwicklung neuer Werk-und Arbeitsstoffe eine *Arbeitsplatzbesichtigung* nicht zu umgehen sein. Die *Arbeitsmethodik* selbst erstellt häufig erst die massiven Expositionsbedingungen, die zur Entstehung eines professionellen allergischen Asthma führen, wie etwa die Verwendung von Spritzpistolen beim Auftragen von Farben, oder die Verstäubung von Arbeitsstoffen durch Düsen z. B. im Druckereigewerbe, oder die massive Staubentwicklung an den verschiedenartigsten Arbeitsplätzen, z. B. in den Schleifereien der Holzindustrie, in Verlade—und Lagerräumen,

in Silos, in Kardenräumen, in der Jute-und Hanfindustrie, in landwirtschaftlichen Betrieben, in Mühlen-und Bäckereibetrieben und vielen anderen Industriezweigen. In Anbetracht dieser besonderen Expositionsverhältnisse, die durch das Berufsmilieu geprägt sind, hat *Hansen* daher mit Recht das professionelle allergische Asthma als eine "aufgezwungene" Sensibilisierung deklariert. Hieran vermag auch die Tatsache nichts zu ändern, dass die konstitutionell leicht Sensibilisierbaren der massiven professionellen Exposition besonders rasch und schnell anheimfallen. Dies spricht nicht, wie so häufig gedeutet, gegen sondern vielmehr *für* den erstrangigen Stellenwert, der dem expositionellen Faktor für die Entstehung des professionellen allergischen Asthma zukommt.

Neben der Exposition ist die oft vorhandene hohe *Antigenpotenz* vieler Arbeitsstoffe für das Zustandekommen des professionellen allergischen Asthma von besonderer Bedeutung. Sie drückt sich aus in der graduellen Ausprägung des *Sensibilisierungsgrades,* der sich durch die sog. "Hauttitration", d. h. Intracutantestung in steigenden Antigenverdünnungen (meist Zehnerpotenzen) zur Darstellung bringen lässt. Bei verschiedenen bekannten professionellen Antigenen sind Hauttitrationswerte von 1:100000 an aufwärts bis zu 1:10 Milliarden (10^{-10}) beobachtet worden, -ein Umstand, der bei der Diagnostik zur Vermeidung von Schockzwischenfällen der Berücksichtigung bedarf. Ich nenne nur einige solcher Antigene: Gummi arabicum, Rohkaffeebohnenstaub, Baumwollsamen und Leinsamenstaub, die verschiedensten Insektenstäube un tierische Antigene wie z. B. Heuschrecken, Küchenschaben, Mehlkäfer und-Motten, Milben, Askarisantigen, Schlangengiftantigen, Muschelschalenantigen, ferner verschiedene pflanzliche und Futtermittelantigene, Soja, Luzerne, Lykopodium und viele andere mehr. Wichtiger jedoch als die graduelle Ausprägung einer Sensibilisierung erscheint mir für die Charakterisierung eines Stoffes als professionelles Antigen die Ermittlung des sog. *Sensibilisierungsindex.* Hierunter versteht man die prozentuale Anzahl der Sensibilisierten zur Gesamtzahl der Exponierten. Er beträgt z.B. bei der Rohseidenindustrie etwa 26%, bei den Mehlberufen (je nach Arbeitsverhältnissen) zwischen 26 und 44%, für das Gummi arabicum zwischen 30 und 55%, bei den berufsbedingten Insektenallergien meist über 50% bei Lycopodium (Gummiindustrie) ca. 20%. Als gemeinsamer klinischer Ausdruck von Sensibilisierungsindex und Antigenpotenz ist der bei einer Reihe von professionellen Antigenen anzutreffende "kollektive Befall" unter der exponierten Arbeiterschaft, ja u.U. sogar der Umwohnerschaft anzusehen,—sog, "endemisches Asthma"—wie uns das Beispiel der Ricinusbohnenstauballergie lehrt.—Systematische Untersuchungen zur Ermittlung des Sensibilisierungsindex stehen leider für viele Berufsgruppen und Industriezweige noch aus, bilden aber für die Zukunft eine wichtige Grundlage für die Beurteilung der versicherungsrechtlichen Zusammenhänge.

Der *Antigenkatalog* der professionellen Antigene ist so umfangreich, dass eine Einzelaufzählung hier nicht erfolgen soll. Einen Überblick über die hauptsächlichen *Mischantigene* sowie einige besonders wichtige berufliche *Solitärantigene* soll nachfolgende Tabelle vermitteln:

STAUBE UND AEROSOLE ALS HÄUFIGE BERUFSANTIGENE

Mischantigene	*Solitärantigene*
Haarstaub	Rohkaffeebohnenstaub
Mehl-Kleiestaub	Baumwollstaub
Federnstaub	Insektenstaub
Polsterstaub	Ricinusbohnenstaub
Drogen- u. Medikamentenstaub	Rohseidenstaub
Blütenstaub	Gummi arabicum-Staub
Kosmetikastäube und -aerosole	
Futtermittelstaub	
Schimmelpilzsporenstaub	
Holzstaub	
Duftstoffaerosole	

Die Gegenüberstellung von Mischantigenen zu Solitärantigenen begründet sich durch die Tatsache, dass bei den sog. Mischantigenen häufig *larvierte* Zusätze und *obligate* Verunreinigungen zu einer professionellen Sensibilisierung führen können, was bei der Diagnostik Berücksichtigung finden muss. Darüber hinaus ist im Schrifttum der letzten Jahre meist an Hand von Kasuistiken über professionelle inhalative Sensibilisierungen gegen folgende Antigene berichtet worden, von denen ich nur die wichtigsten hier aufzählen möchte:

1. *Metalle*: Platin, Chrom, Vanadium, Beryllium, Nickeltetracarbonyl und Kobalt;

2. *Tierische Antigene*: Schlangengift, Haarstäube von Laboratoriumstieren (Meerschweinchen, Hamster, Ratten, Mäuse), Küchenschaben, Mehlkäfer, Mehlmotten, Milben, Ascaris, Hirschhaare, Hasenhaare, Muschelschalen (Knopfindustrie);

3. *Pflanzliche Antigene*: Luzerne, Soja, Lykopodium (Gummiindustrie), Narzissen- und Tulpenzwiebeln, Bekunistee;

4. *Chemikalien*: Insektizide, Naphthochinon, Epoxydharze, Formaldehyd, Turbinenöl (Zahnarzt).

Diese Liste macht keineswegs Anspruch auf Vollständigkeit und bedarf einer ständigen Ergänzung. Zugleich ist sie ein eindeutiger Beweis dafür, dass die Zahl derartiger "aggressiver" Antigene keineswegs "gering" ist, wie fälschlicherweise in der Literatur häufig behauptet wird.

Zum *klinischen Bild* und zur *Diagnostik* des professionellen allergischen Asthma nur einige wenige Bemerkungen. Die Entwicklung des klinischen Bildes als Ausdruck einer stattgehabten und nunmehr aktuellen Sensibilisierung gegenüber dem antigenen Arbeitsstoff zeigt sehr häufig die bekannten Prodromi und "Äquivalente" an den Bindenhäuten der Augen sowie den Schleimhäuten im Nasen-Rachen-Raum und den oberen Luftwegen. Diese Erscheinungen können allein oft Monate oder Jahre hindurch bestehen bleiben. Es ist charakteristisch, dass sich in

der überwiegenden Mehrzahl der Fälle erst *langsam* zunehmend ein Beklemmunsgefühl auf der Brust mit Anstrengungsdyspnoe sowie mit spärlichem Giemen und Brummen und geringen Mengen zähflüssigen Auswurfs entwickelt. Nur relativ selten gibt sich die Ausweitung der allergischen Entzündung auf die tieferen Luftwege durch einen akuten Paroxysmus kund, sondern schleichen sich die asthmatischen Symptome langsam über das Stadium der "allergischen Bronchitis" ein. Die anfängliche Bindung an Arbeitszeit und Arbeitsplatz sowie die rasche Erholung bei Meidung der Betriebsstätten in den Mittags- und Nachtstunden sowie übers Wochenende verschwinden mehr und mehr. Während im Beginn meist innerhalb von 1-2 Stunden nach Verlassen des Arbeitsplatzes Beschwerdefreiheit durch Verschwinden der Äquivalente charakteristisch ist, nimmt im Laufe von Monaten und Jahren die asthmatische Dyspnoe langsam zu. Das erscheinungsfreie Intervall wird kürzer, und kürzer, die Nachtruhe ist gestört, und erst nach mehrtägiger Arbeitskarenz tritt wieder Beschwerdefreiheit ein, bis es über jahrelangen Verlauf zur Entwicklung eines "chronischen Dauerasthma" mit seinen charakteristischen Sekundärfolgen kommt. Die Darstellung dieser fast *regelhaften* Entwicklung über das Stadium der asthmatischen Bronchitis ("Subasthma" nach *Hansen*) ist zu beachten, insbesondere im Hinblick auf die *unterschiedliche* Interpretation des Krankheits begriffes "Bronchialasthma", als eines ausschliesslichen oder vorwiegenden Anfallsleidens. Denn gerade beim professionellen allergischen Asthma treffen wir bei den einzelnen Kranken einen oft recht unterschiedlichen Zeitraum vom Beginn der Arbeitszeit (Exposition) bis zum Auftreten subjektiver Atembeschwerden. In zahlreichen Fällen beginnen die ersten Beschwerden erst 2-4 Stunden, seltener 6 Stunden nach Arbeitsbeginn. Manchmal stellt sich die Dyspnoe sogar erst *nach* Verlassen des Arbeitsplatzes ein. Dieser klinische Hinweis erscheint mir wichtig, um nicht voreilig die Frage einer möglichen professionellen Entstehung zu verwerfen. Die Latenzzeit hängt wahrscheinlich -worauf als erster *Tiffeneau* hingewiesen hat- mit einem Reizschwellenproblem zusammen und entspricht der sog. "verzögerten" Reaktion der Bronchialschleimhaut bei der experimentellen Provokationsprüfung mit Antigen-Aerosolen.

Im Zentrum der klinischen *Diagnostik* steht der Nachweis der Allergiebedingtheit durch die bekannten klinischen Kriterien allergischer Reaktionsweise. In Bezug auf das professionelle allergische Bronchialasthma möchte ich hier nur einen mir wichtig erscheinenden Gesichtspunkt herausgreifen und betonen, dass trotz hinweisender Anamnese sowohl ein positiver cutaner Antikörpernachweis wie aber auch ein negatives Ergebnis für die sozialrechtliche Beurteilung in der Frage einer eventuellen Entschädigungspflicht häufig nicht genügen. Denn die Entscheidung dieser Frage kann auf Grund eigener, sehr umfangreicher Erfahrungen und einer ausgedehnten Gutachtertätigkeit *nur* durch die Anstellung und den Ausfall von *Provokationsproben* an der Bronchialschleimhaut mit genügender Sicherheit abgeklärt werden. "Falsch positive" Hautreaktionen im Sinne einer "non clinical allergy" Tuft's wie aber auch "falsch negative" Hautreaktionen sind ein häufiger Anlass zu

Fehlurteilen. Welcher dynamischen Ventilationsgrösse man sich bei der Registrierung der durch Antigen-Aerosole provozierten asthmatischen Dyspnoe bedient, ist dabei von untergeordneter Bedeutung. Um die Aktualität des fraglichen Antigens zu beweisen, kann man sich meist mit der fortlaufenden Kontrolle *einer* Atemgrösse (Vitalkapazität, Atemstoss, Atemgrenzwert, etc.) begnügen.

Abschliessend einige wenige Bemerkungen und Richtlinien für die Prüfung und Beurteilung *versicherungsrechtlicher* Ansprüche. Diese richten sich:

1. nach dem Vorkommen des Antigens im beruflichen und ausserberuflichen Milieu;

2. nach der Ausschaltbarkeit des Antigens.

Was die Verbreitung des Antigenes und sein Vorkommen im beruflichen wie im natürlichen Lebensraum anbetrifft, haben sich die von *Spain* und *Fontana* aufgestellten Grundsätze für uns als praktikabel erwiesen. Die Anerkennung des professionellen Zusammenhanges macht keine Schwierigkeiten, wenn

1. die Antigen-Exposition auf den Arbeitsplatz beschränkt ist, und keine ausserberuflichen Kontaktmöglichkeiten bestehen;

2. der nichtprofessionelle Antigenkontakt grössenordnungsmässig weit unter der massiven Exposition am Arbeitsplatz liegt, wie z. B. bei einer Arbeiterin in einer Bettenfabrik.

Zu erheblichen Schwierigkeiten in der Beurteilung der professionellen Zusammenhangsfrage kann es jedoch kommen, wenn

3. das professionelle Antigen sowohl am Arbeitsplatz, wie im natürlichen Lebensraum sehr reichlich vorhanden ist, wie z. B. bei einem Staubsaugervertreter oder Gebäudereiniger mit einer Hausstauballergie.

In solchen Fällen kann nur ein sorgfältiges Abwägen unter Berücksichtigung der Entstehung, des Verlaufes sowie des Ausfalles von Karenz und Re-Expositionsproben am Arbeitsplatz eine Wahrscheinlichkeit in der Beurteilung der Zusammenhangsfrage erreichen.

Was die Höhe des *Entschädigungsanspruches* bei Bejahung der Zusammenhangsfrage anbetrifft, so ist die Verbreitung des professionellen Antigenes in der verschiedenen Berufssparten bzw. seine *Ausschaltbarkeit* ein oft richtunggebender Masstab. So bedarf gerechterweise eine professionelle Sensibilisierung gegen ein relativ selten vorkommendes und leicht ausschaltbares Antigen wie z. B. Rohkaffeebohnenstaub, das oft durch einen einfachen Arbeitsplatzwechsel innerhalb des Betriebes zu umgehen ist, einer geringeren prozentualen Bewertung des Entschädigungsanspruches, als eine z. B. in der Gährungsindustrie erworbene professionelle Sensibilisierung gegen Schimmelpilzsporen, -ein ubiquitäres Antigen, das sowohl an vielen anderen Arbeitsplätzen, wie aber auch im ausserberuflichen Milieu zur Auslösung von Beschwerden führt und durch einen Arbeitsplatz- oder Berufswechsel sehr viel schwerer, u. U. gar nicht zu umgehen ist.

Nach diesen wenigen *grundsätzlichen* Bemerkungen über die Rolle der berufsbedingten Sensibilisierung für die Entstehung eines profes-

sionellen Bronchialasthma darf ich abschliessend die eigentlichen *gestaltenden* Faktoren noch einmal hervorzheben:

Es sind die durch das Berufsmilieu bedingte *Expositionsgrösse* sowie die häufig anzutreffende *hohe Antigenpotenz* der Arbeitsstoffe. Im Gegensatz zu einer "spontanen" Allergie entsteht erst durch ihr Zusammenwirken jenes besondere *Krankheitspotential,* das (nach *Hansen*) auch die Tragfähigkeit der normalen, d. h. der familiär *unbelasteten* Konstitution zu übersteigen vermag.

SYMPOSIUM:

FRECUENCIA DE FACTORES ETIOLOGICOS DEL ASMA BRONQUIAL EN VARIOS PAISES

FREQUENCY OF AETIOLOGIC FACTORS IN BRONCHIAL ASTHMA IN THE DIFFERENT COUNTRIES

FREQUENCE DES FACTEURS ETIOLOGIQUES DE L'ASTHME BRONCHIQUE DANS DIFFERENTS PAYS

DIE HAUFIGKEIT ÄTIOLOGISCHER FAKTOREN BEIN BRONCHIALASTHMA IN VERSCHIEDENEN LANDERN

L. Herráiz Ballesteros.

Z. Bernath.

L. E. Betetta.

H. Colldahl.

J. L. Cortés.

M. Damas Mora.

Z. Erikson-Lihr.

E. Fuchs.

B. Fust.

L. N. Gay.

I. Glazer.

J. M. Jamar.

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J. Leger.

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G. Moreno Valero.

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R. Ordman.

A. Pérez Lozano.

W. J. Quarles van Ufford.

M. Sánchez Medina.

P. Sangiorgi.

V. Spoujitck.

P. Ward Farmer.

FACTORES ETIOLOGICOS DEL ASMA BRONQUIAL EN LA ARGENTINA

L. HERRAIZ BALLESTERO, con la colaboración de los doctores J. BACCIGALUPPI, R. BRUJIS, E. MATHOV y C. RODRÍGUEZ FONTELA.

Los datos que a continuación se analizan proceden de revisión de historias clínicas, especialmente preparadas para la estadística, de enfermos que concurrieron al consultorio externo desde 1943 hasta 1963.

Estas historias han sido separadas en dos grupos, según la zona de que procedía el enfermo. Los estudios sobre polinosis y otras enfermedades alérgicas, publicadas en 1938-1943 (HERRÁIZ) demostraron que la parte más densamente poblada del país está dividida en dos zonas, donde los factores etiológicos difieren considerablemente:

Zona I, situada al este de la Isoyeta 500, llamada pampa húmeda, donde está radicada la mayor parte de la población del país y las lluvias superan los 500 mm. por año.

Zona II, situada al oeste y al sur de la misma línea, llamada pampa seca, cuya densidad demográfica es menor y las lluvias escasas.

Aunque en ambas zonas hay costa oceánica, lagos, ríos y montañas, estos "enclaves climáticos" no hacen desaparecer las diferencias de clima, flora, suelo y comportamiento de las enfermedades alérgicas que distinguen una zona de la otra.

Hay una tendencia exagerada a atribuir el beneficio de cambio de clima a un simple efecto de disminución alérgica. En muchos casos hemos podido comprobar que la mejoría no se debe al alejamiento de excitantes.

En general, los enfermos de la pampa húmeda mejoran al trasladarse a la pampa seca, cualquiera que sea el tipo de asma que padezcan. Los de la pampa seca no mejoran al trasladarse a la pampa húmeda, salvo que sean polínicos puros.

Se consignan a continuación los datos obtenidos en Buenos Aires (pampa húmeda más litoral fluvial), Mar del Plata (pampa húmeda más costa oceánica) y Bahía Blanca (pampa seca con costa oceánica, a menos del 1.° paralelo al sur del Mar del Plata sobre la misma costa atlántica), cuya dieta y forma de vida son idénticas. Además la temperatura y cambios atmosféricos de Mar del Plata son muy semejantes a los de Bahía Blanca.

La proporción en que se presentan las manifestaciones alérgicas no respiratorias es aproximadamente la misma en las tres localidades.

Pero la proporción en que se presenta la polinosis es diez veces mayor en Bahía Blanca, y al mismo tiempo disminuyen considerablemente la coriza y el asma de otras etiologías. La constancia de otras manifestaciones que acompañan al asma, como es el eczema y el prúrigo, da la impresión de que la polinosis ha desplazado las demás etiologías, incluso la microbiana.

TABLA I

	BUENOS AIRES (Pampa húmeda en litoral fluvial)	MAR DEL PLATA (Pampa húmeda en litoral oceánico)	BAHIA BLANCA (Pampa seca en litoral oceánico)
Coriza polínica	6,9	3,5	73,5
Coriza no polínica	62,5	60,0	11,0
Asma polínica	0,5	0,0	46,3
Asma bacteriana y mixta.	54,2	62,2	4,4
Urticaria	28,4	31,4	31,6
Eczema	19,1	20,0	22,0
Prúrigo	8,8	11,4	11,0
Jaqueca	15,1	15,0	16,9

Se han analizado 3.939 historias que proceden de diferentes grupos de población, por lo que no se funden en una tabla única.

A pesar de lo heterogéneo de la procedencia de los casos, los resultados son absolutamente concordantes, razón por la que en este resumen se reproducen los datos de uno solo de nosotros (HERRÁIZ).

Estos se han obtenido sobre 1.706 historias. De ellas, 6 corresponden a casos de asma sintomática producida por tumor endobronquial (1), tumor mediastínico (2), complejos primarios tuberculosos (2) y quiste hidatídico (1). Lo exiguo de su número no refleja la frecuencia real de este tipo de asma, sino el hecho de que a nuestro consultorio especializado llegan sólo enfermos alérgicos.

En las tablas II y III se reúnen los porcentajes obtenidos sobre las 1.700 historias restantes de asma primaria, que se descomponen como sigue:

	Hombres	Mujeres	Total
Zona I	560	796	1.356
Zona II	190	154	344
	750	950	1.700

El término de asma alergénica se aplica a aquellos casos que no sólo tienen reacción cutánea francamente positiva, sino a los que la eliminación y la exposición producen con seguridad sus síntomas.

El término de asma bacteriana se aplica a aquellos casos cuyos síntomas están en clara relación con un proceso infeccioso cuyas características más frecuentes son las del catarro habitual descendente y en los que el tratamiento con antibióticos produce una modificación por lo menos parcial de los síntomas. Incluye también aquellos enfermos en que por la antigüedad y reiteración de sus accesos debieran más bien calificarse de inflamatorios de etiología desconocida.

El término de asma mixta se aplica a aquellos casos de asma bacteriana en que la exposición a otros alergenos es capaz de agravar los síntomas, demostrándose también reacciones cutáneas francamente positivas a los mismos.

Damos así a cada uno de estos términos el mismo contenido que su autor, el profesor CARLOS JIMÉNEZ DÍAZ.

Si bien la distinción entre el tipo alergénico y los demás ha sido fácil, no lo fue tanto clasificar muchos casos de asma mixta, que suponemos son una transición evolutiva hacia el asma no alergénica, una etapa de esa evolución del enfermo que empieza siendo alergénico para convertirse finalmente en asma infeccioso y/o inflamatorio de etiología desconocida (asma intrincado).

Dentro de cada tipo de asma se ha considerado la importancia causal de los alimentos, del polen, del polvo de casa, de los restantes inhalantes domésticos agrupados en una sola cifra, concediéndoles importancia solamente cuando se reunían por lo menos dos de estas circunstancias: prueba cutánea positiva, experiencia del enfermo o prueba de provocación.

La sensibilidad a los gérmenes de localización bronquial e intestinal se valoró por la aparición de reacción cutánea tardía infiltrativa de más de 4 cm. de diámetro a una vacuna obtenida de la flora de esputos asmáticos o de materias fecales, respectivamente, ambas de composición constante.

Se consignan a continuación en las mismas tablas el porcentaje de representantes del sexo femenino que componía cada uno de los tres tipos de asma y dentro de la cifra absoluta que correspondía al sexo, el porcentaje de los que empezaron a padecer sus síntomas antes de los diez años en cada uno de los tipos de asma considerados. A cantinuación figuran los mismos datos correspondientes al sexo masculino.

Como suponíamos, los resultados obtenidos en la zona I difieren de los obtenidos en la zona II, por lo que se comentan por separado.

Los datos obtenidos de los 1.356 enfermos de la zona I pueden resumirse en las siguientes observaciones:

1.ª El asma alergénica constituye sólo la quinta parte del total de los casos examinados (21 por 100).

2.ª El asma bacteriana (32 por 100) y la mixta (46 por 100) (que debe ser considerada también como dominantemente microbiana), constituyen la mayor parte de los casos (78 por 100).

3.ª Los casos de asma alergénica responden a la eliminación de los excitantes correspondientes, pero en casi la quinta parte de los casos las reacciones cutáneas a gérmenes son intensas.

TABLA II

	Tipo	Alimentos	Polen	Polvo de casa.	Inhalantes domésticos.	Gérmenes bronquiales.	Gérmenes intestinales	Sexo femenino total	Sexo femenino, 0 a 10 años	Sexo masculino total	Sexo masculino, 0 a 10 años	Herencia (Antecedente de)
Zona I												
A. Alerg.	21	33	6	70	64	23	25	56	47	43	74	45
A. Bacte.	32	0	—	32	30	70	78	60	42	40	76	39
A. Mixta	46	17	—	50	51	71	80	58	41	42	75	42

TABLA III

Zona II												
A. Alerg.	46	*	53	24	21	16	21	38	20 ***	62	80 ***	32
A. Bacte.	30	*	9 **	32	30	69	70	33	55 ***	66	80 ***	39
A. Mixta	24	*	10 **	32	31	68	71	32	48 ***	68	75 ***	40

(*) Faltan datos para llenar las condiciones requeridas (test +, anamnesis + y provocación +).
(**) Reacciones cutáneas positivas en 12 % de la población "asintomática".
(***) De 0 a 16 años.

4.ª En el asma microbiana y en el asma mixta disminuye la frecuencia con que pueden comprobarse sensibilidad a polen, polvo de casa y otros inhalantes, y aumenta considerablemente la sensibilidad a los gérmenes bronquiales e intestinales.

5.ª En los tres tipos de asma estudiados la incidencia es mayor en el sexo femenino, aunque la aparición de los síntomas es más tardía en la mujer, por esta razón, entre cero y diez años hay una neta disminución con respecto a los varones.

6.ª En el sexo masculino el asma, que es menos frecuente que en la mujer, aparece más precozmente (74/76 por 100 de los casos antes de los diez años).

7.ª En los tres tipos de asma, los antecedentes familiares son igualmente frecuentes (39/45 por 100), excepción hecha del asma alergénica de la zona II.

Los datos obtenidos de los 344 enfermos radicados en la zona II difieren de los anteriores en los siguientes puntos:

1.° La polinosis es diez veces más frecuente que en la zona I y a beneficio de ella aumenta la etiología alergénica.

2.° Hay una clara disminución de la proporción de casos mixtos, permaneciendo invariable la frecuencia del asma infecciosa.

3.° Se acentúa la precocidad de aparición de los síntomas del sexo masculino respecto al femenino.

4.° En ambos sexos la aparición de los síntomas se retarda, por lo que ha sido necesario computar los casos aparecidos en el lapso 0-15 años de edad en lugar del de 0-10 años, que se utilizó en la zona I para expresar la precocidad de los síntomas.

5.° La frecuencia de los antecedentes familiares es menor entre los casos de asma alergénica de la zona II, probablemente por la mayor proporción de polinósicos que entre ellos se encuentra.

FRECUENCIA DE FACTORES ETIOLOGICOS DEL ASMA BRONQUIAL EN CHILE

Z. BERNATH, E. DÍAZ, E. CARRASCO, R. GUZMÁN, J. GRAU. e I. MUCHNICK (*).

Esta presentación contiene las observaciones sobre más de 30.000 casos de asma bronquial observados en los últimos diez años en los principales centros especializados en Santiago de Chile.

Desde el punto de vista de la etiología, hemos hecho la división en dos grupos grandes: 1.°, asma bronquial de evolución continua, y 2.° asma bronquial de presentación estacional. En el primer grupo los factores etiológicos principales en orden de importancia son las infecciones bronquiales, con la consiguiente alergia microbiana; las alergias a hongos aerógenos; polvo de habitación y diferentes otros inhalantes que se enumeran.

En el grupo del asma bronquial de presentación estacional, el factor etiológico lo constituyen, en orden de importancia, los siguientes pólenes: plátano oriental, gramíneas, setevenas, los cardos, las romasas, etcétera.

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FRECUENCIA DE FACTORES ETIOLOGICOS DEL ASMA BRONQUIAL EN EL PERU (*)

LUIS E. BETETTA (**)

En el frondoso árbol etiológico del asma bronquial hay factores conocidos universalmente y otros que son peculiares de cada país.

Así, en el Perú estos factores están ligados íntimamente a su geografía y a las costumbres y ocupaciones de sus habitantes.

Sólo por homologar datos con los demás colegas consignaremos los factores étnicos, de edad, hereditarios, endocrinos, de malformaciones o infecciones en el tracto respiratorio, metabólicos (hidratos de carbono), psíquicos, inhalantes, alimenticios, geo-climáticos, bacterianos y ocupacionales (Cuadro 1.).

Los treinta años de observaciones sobre el asma bronquial nos dan credenciales para aunar a la enumeración de los factores etiológicos la sistemática de presentación y evolución de tal síndrome.

Sentamos como premisa que el asma bronquial no se presenta en forma exabrupta. A su presentación anteceden otros síndromes, como rinitis, traqueítis, bronquitis asmatiforme, bronquitis asmática, prúrigo, hiperqueratosis folicular, urticaria, eczema, reumatismo, gastritis, gastroenteritis, colon irritable, síndrome disentérico, síndromes que a su vez pueden reemplazar al asma bronquial (Cuadro 2.).

Herencia.—Es indiscutible que el 80 por 100 de los casos de asma bronquial observados en el Perú son tributarios del abono hereditario unilateral o bilateral.

Este factor hereditario presenta las siguientes características:

1) Aparición del síndrome asmático en forma precoz o de un equivalente alérgico: traqueobronquitis, bronquitis asmatiforme o procesos dérmicos, como prúrigo o eczema, ligados a la alimentación (leche) o vestimenta (ropones de lana o algodón), respectivamente.

2) La confluencia bilateral de antecedentes alérgicos hereditarios condiciona la precocidad de la aparición del síndrome (a la semana del nacimiento) y hace más severa su evolución, casi con pronóstico de perennidad (Cuadro 3.).

3) Cuando el abono hereditario es *unilateral,* la herencia del síndrome, por lo general, es cruzada al sexo y al físico de los padres. Si el padre es asmático, son las hijas con parecido fisonómico a él que here-

(*) Observaciones a través de treinta años hechas por los miembros de la Sociedad Peruana de Alergia, Expositor: Luis E. Betetta.

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CUADRO 1

Factores etiológicos generales del asma bronquial

Raza	
Edad	
Herencia	Unilateral Bilateral
Endocrinos	Menarquía-Menopausia. Hipófisis. Tiroides Suprarrenal.
Malformaciones e infecciones en el tracto respiratorio	Vegetaciones adenoides. Hipertrofia de cornetes. Amigdalitis. Sinusitis frontal y maxiliar.
Metabólicos:	Hidratos de carbono.
Psíquicos.	
Inhalantes.	
Alimenticios.	
Geo-climáticos.	
Bacterianos.	
Ocupacionales.	

CUADRO 2

Síndromes vicariantes que anteceden o suceden al asma bronquial
(Observaciones entre 1933 a 1963 sobre 35.000 pacientes)

Respiratorios	*Dermatológicos*	*Otros*
Rinitis.	Prúrigo.	Reumatismo.
Traqueitis.	Hiperqueratosis folicular.	Gastritis.
Bronquitis asmatiforme.	Urticaria.	Gastroenteritis.
Bronqutis asmática.	Eczema.	Colon irritable.
		Síndrome disentérico.

Tiempo mínimo: De uno a cinco años, antes o después del asma bronquial.
Tiempo máximo: De uno a diez años, antes o después del asma bronquial.

dan el asma; a la inversa, si la madre tiene antecedentes de asma, los hijos varones con parecido fisonómico a ella heredan el síndrome.

4) Sólo en los casos de herencia se presenta precozmente, entre uno y tres años, el síndrome de *rinitis alérgica*. Cuando no hay antecedentes alérgicos, este síndrome aflora por lo general a partir de los siete años. Hacemos salvedad en la alergia bacteriana, que puede desencadenar rinitis y asma en los primeros meses. Otro tanto puede ocurrir con las malformaciones nasales.

5) En los niños el asma ocasionado por alergenos alimenticios se presenta entre uno y cuatro años, desapareciendo generalmente a los cinco años.

ASMA BRONQUIAL EN RELACIÓN CON LA GEOGRAFÍA, RAZA, EDAD Y OCUPACIONES EN EL PERÚ.

El asma bronquial puede presentarse en cualquier período de la vida y en cualquier lugar de un país. Sin embargo, debemos hacer salvedades en relación con el Perú. (Fig. 1.).

Geográficamente, el Perú está dividido longitudinalmente por la Cordillera de los Andes en tres regiones: Costa, Sierra y Montaña o Selva. De esta topografía dependen sus climas y, por ende, la incidencia del asma bronquial. El calendario peruano marca cuatro estaciones: primavera (23 de septiembre a 23 de diciembre), verano (23 de diciembre a 23 de marzo), otoño (23 de marzo a 23 de junio), invierno (23 de junio a 23 de septiembre). Sin embargo, sólo percibimos dos estaciones: invierno (mayo a octubre) y verano (octubre a mayo). Estas dos estacio-

CUADRO 3

Asma bronquial y otros síndromes en relación con la herencia y edad.
(Observaciones entre 1933 a 1963 sobre 35.000 pacientes)

Herencia			
Unilateral	Bilateral	Edad	Síndromes
Sí		1 a 2 años	Rinitis - ASMA - Prúrigo.
	Sí	7 a 60 días	ASMA BRONQUIAL - Prúrigo Eczema.
No	No	7 años	Rinitis - ASMA BRONQUIAL.
No	No	15 a 45 años	Rinitis - ASMA BRONQUIAL.
No	No	55 a 65 años	ASMA cardíaca.
Mujeres		38 a 45 años	ASMA ENDOCRINA.

OBSERVACIONES INTERESANTES

1.ª En el asma bronquial la herencia se manifiesta *cruzada* en relación con el sexo de los padres: de MADRES ASMATICAS son los *hijos* los predispuestos al síndrome; de PADRES ASMATICOS son las *hijas* las que heredan la predisposición. El porcentaje no escapa del 80 por 100.

2.ª Las rinitis y asma bacterianas suelen presentarse en niños de días o meses por malformaciones nasales o infecciones rinofaríngeas, aun sin antecedentes alérgicos hereditarios.

CUADRO 4

Climas y humedad en costa, sierra y selva del Perú y la incidencia del asma bronquial.

Clima:	Costa	Sierra	Selva
Verano	Octubre a mayo	Abril a octubre	Abril a septiembre
Invierno	Junio a septiembre	Noviemb. a marzo	Octubre a marzo, con grandes lluvias alternadas con sol tropical
Humedad	78 % a 99 %	20 % a 30 %	48 % a 75 %
Asma y porcentaje	20 %	2 %	4 %
Alergenos más frecuentes	Inhalantes	Pelos de auquénidos	Flora micótica y parasitosis

CUADRO 5

Geografía y asma bronquial en el Perú

(Observaciones entre 1933 a 1963 sobre 35.000 pacientes)

Edad	Costa Porcentaje	Sierra Porcentaje	Montaña Porcentaje
1 a 5 años	3 %	0 %	1 %
5 a 15 años	10 %	1 %	2 %
15 a 45 años	20 %	4 %	6 %
45 a 60 años	8 %	2 %	4 %

nes son especialmente marcadas en la costa del Perú. *La temperatura máxima* en verano es de 28° y la de invierno baja hasta 11°. *No hay lluvias profusas*, sino una *llovizna que contribuye a la saturación del ambiente por una humedad que se constata durante todo el año* entre 78 por 100 a 99 por 100, *aun en la época de verano,* por lo que *entre los meses de septiembre o marzo* (primavera y verano) esta humedad y el calor fomentan la pululación de hongos ambientales a determinadas horas, según observación del doctor PEDRO VARGAS MORALES, del Instituto de Investigaciones Alérgicas del Perú.

La incidencia del asma bronquial en la Costa es mayor en verano que en invierno, seguramente por la razón expuesta arriba. A esto se agrega el hecho de que siendo la Costa una zona industrializada, además de los alergenos comunes, como polvo de casa, hongos, etc., etc., se añaden los elementos alergizantes de cada industria, como la de harina de pescado, detergentes, tejidos, tabaco, construcción civil, como puede verse en los cuadros correspondientes. El polen no da síndromes alérgicos de apre-

ciación clínica. Sólo agrava los accesos de asma bronquial, de acuerdo a nuestras observaciones. La razón de no presentarse el cuadro típico de polinosis parece consistir en que la humedad rompe la cápsula de los gránulos de polen haciéndoles perder su antigenicidad.

Mientras en la Costa hay verano, en la Sierra (zona a partir de los

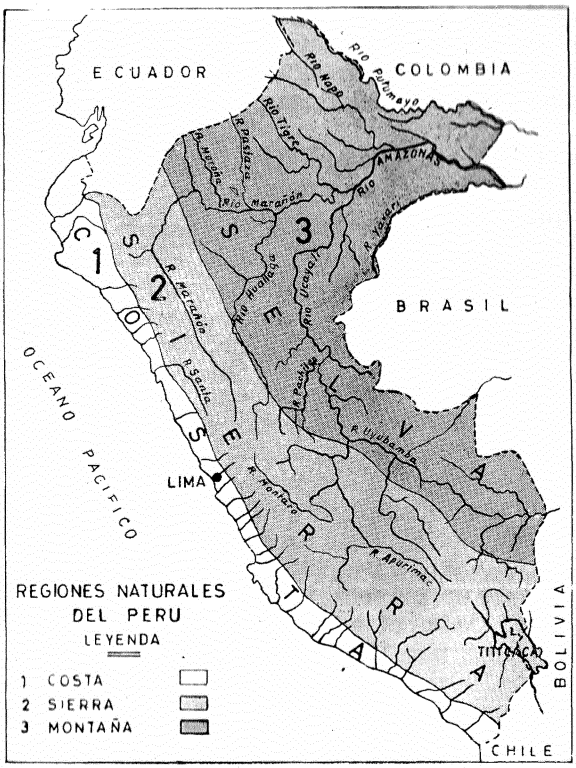

Fig. 1.—Mapa del Perú.

50 km. de la Costa) hay grandes lluvias, alternadas con escasos días de sol. Es importante hacer notar que en la Sierra la humedad es casi nula y la incidencia de asma bronquial no existe, sino en un porcentaje insignificante. Esta nulidad en la incidencia del asma bronquial determina que a los asmáticos con crisis subintrantes o "estado asmático" se les mande de la Costa a la sierra, y se constata que los accesos van desapareciendo desde los 40 km. de la costa.

En la Sierra, el invierno comienza en noviembre y continúa a marzo, presentándose en abril un clima templado alternado con sol y grandes heladas que se turnan con copiosas lluvias de agosto a octubre. Debido al frío seco de la altura, la Sierra aparentemente está desprovista de alergenos inhalantes como polvos de casa, y las pruebas intradérmicas con el extracto de polvos de casa de la Costa en los habitantes de la Sierra son negativas.

Debemos resaltar que entre los inhalantes de la Sierra que motivan

asma bronquial están los pelos de ciertos auquénidos, como de la llama, alpaca, vicuña.

Las colonias de hongos en la Sierra son escasas y no tienen el poder antigénico de los hongos de la Costa.

En la montaña o Selva el clima es proyección del clima de la Sierra, con alternativas de sol tropical y lluvias torrenciales. En esta región el asma bronquial se presenta en porcentaje bajo, casi despreciable, en comparación con la incidencia en la Costa. Es de suponer que la humedad contribuya a la eclosión del asma bronquial de etiología parasitaria y micótica.

En la Costa el porcentaje de asmáticos es del 20 por 100, en tanto que en la Sierra tal porcentaje es escaso, no más del 2 por 100, y con la particularidad de que no existe el asma en los niños, aun con antecedentes hereditarios alérgicos. Debemos resaltar también que los adultos y niños que no han tenido asma bronquial en la Sierra, al llegar a la Costa fácilmente se alergizan y en ellos aflora el asma bronquial, especialmente en los obreros que manipulan elementos sensibilizantes, como harina de pescado.

En la Montaña el porcentaje de asma no pasa del 4 por 100, incriminable a parasitosis intestinal y flora micótica.

Raza.—En cuanto a las razas, en relación con el asma bronquial, el porcentaje mayor corresponde al tipo *mestizo* (cruce de blancos con indios); este porcentaje, 14 por 100, corresponde a una población que de su total (11.000.000) corresponde el 70 por 100 a la *raza mestiza.* La *raza blanca,* que representa un 20 por 100 de la población total, rinde tributo al asma bronquial en un porcentaje del 7 por 100. La asiática, que representa el 1 por 100 de la población total, es víctima del asma bronquial en un porcentaje del 2 por 100. Finalmente, la *raza negra,* a pesar de representar un 9 por 100 de la población total, sin embargo, es afectada en proporción mínima por el asma bronquial, apenas el 1 por 100 (Cuadro 6.).

CUADRO 6

Raza y asma bronquial en el Perú

Raza	Porcentaje	Porcentaje.
Blanca	7 %	20 %
Mestiza	14 %	70 %
Asiática	2 %	1 %
Negra	1 %	9 %

Edad.—Al ocuparnos de la herencia hemos enunciado que el asma bronquial se presenta precozmente en los que tienen antecedentes hereditarios uni o bilaterales, apareciendo en los primeros días de vida. Si no hay esos antecedentes, la frecuencia observada es de siete a cincuenta años; pasada esta edad el asma es *cardíaca primaria* o como epílogo del asma crónico.

Es importante hacer notar que en los niños sin antecedentes alér-

gicos hereditarios no hay rinitis ni asma bronquial antes de los cinco o siete años, edades en las que se generan esos síndromes por exposición a los elementos sensibilizantes del ambiente o por labilidad orgánica motivada por infecciones, salvo excepciones.

En las mujeres, en la época de la menopausia, aparecen síndromes alérgicos, como rinitis, asma bronquial o dermatosis que se agravan en el período menstrual, si todavía lo tienen, y desaparecen con la cesación de los síntomas del climaterio.

Asma bronquial de origen ocupacional en el Perú.

La frecuencia del asma bronquial en relación con las actividades laborales la constatamos en la prolija y prolongada investigación que hemos hecho los alergistas peruanos en los diversos centros industriales, que suman 13, y sobre un total de 22.053 obreros. Es indiscutible que para la afloración del síndrome median factores de disposición y exposición del obrero.

En premio al tiempo, señalaremos someramente, y en columnas, el porcentaje de *síndromes alérgicos respiratorios* encontrados en los trabajadores de 13 industrias desde el año 1957 a 1962. (Cuadro 7.)

La gama de síndromes respiratorios vicariantes nos persuade para razonar que ellos anteceden al entronizamiento del *asma bronquial.*

CUADRO 7

Síndromes alérgicos respiratorios de origen ocupacional

Industria	Rinitis	Traqueo-bronq.	Bronq. asmatif.	Bronq. asmat.	Asma br.
Textil	15.5 %	30.6 %	29 %		4.8 %
Construcción civil	19.5 %			23.9 %	3.7 %
Maderera	25 %		31.2 %		43.8 %
Harina de trigo	76 %	18.1 %			5.9 %
Cueros y calzado	46.8 %			20.5 %	5.8 %
Detergentes y jabones	11 %		32.9 %		5.1 %
Peletera	47 %			8.9 %	44.1 %
Tabaco	25.6 %	12.4 %	17.5 %		2.1 %
Harina de pescado	12.7 %		19.7 %		7.9 %
Insecticidas	16.1 %	16.1 %			
Polvillo de menestras y cereales	42.5 %	27.4 %			3.5 %
Avícola	64,3 %				9.6 %
Imprenta	44.4 %			11.9 %	4 %

ALERGENOS MÁS FRECUENTES DEL ASMA BRONQUIAL EN EL PERÚ.

Para ser breves enumeraremos los alergenos más frecuentes en la etiología del asma bronquial en el Perú.

CUADRO 8

INHALANTES

1. Polvos de casa.
2. Relleno de colchón (crin, paja, lana vegetal, fibras de cereales, algodón).
3. Hongos anemófilos.
4. Papel viejo, billetes viejos.
5. Pelos y epitelios de animales (perros, gatos, conejos, caballos, auquénidos (llama, alpaca, vicuña).
5. Cachemira o lana de angora.
6. Lana animal, Chenille.
7. Materiales de construcción civil (cemento, cal, yeso).
8. Tejidos variados.
9. Aserrín de madera (tornillo).
10. Harinas de pescado y trigo.
11. Polvillo de cueros.
12. Tabaco.
13. Plumas de aves.
14. Detergentes.
15. Cosméticos (polvos, talco, perfumes).
16. Plumas de aves.
17. Hongos patógenos.
18. Polvillo de trigo, cereales y menestras.
19. Petróleo y derivados.
20. Pólenes (no dan cuadros clínicos de Polinosis; sólo agravan los síndromes respiratorios).
20. Plásticos y acrílicos y otras diversas industrias.

ALIMENTOS

1. Pescado.
2. Huevo, leche, trigo, carnes y pescados desecados, grasas, aceites, condimentos (pimienta, achote, cominos y otras especerías).
   Arroz, naranja y cítricos.

OTROS FACTORES

1. Endocrinos.
2. Bacterianos.
3. Parasitarios.
4. Drogas.
5. Antibióticos.

Como corolario de los factores etiológicos del asma bronquial en el Perú es necesario hacer una relación de elementos inhalantes, bebidas y alimentos que *sin ser sensibilizantes desencadenan el acceso asmático:* por eso los llamamos *disparadores o reveladores.*

Estos, en el Perú, son los siguientes:

Emanaciones olorosas.

Olor de apio, de perejil, de culantro, de albahaca, de orégano, de ruda, de hierbabuena, de margaritas, de geranio.

Bebidas.

Vinagre, pisco (alcohol de uva), cerveza, vino (especialmente el tinto), chicha de Jora (maíz fermentado), chicha morada (maíz morado fermentado), bebidas gaseosas, champagne.

Alimentos: Frutas.

Gelatinas, anticuchos, cebiche (pescado macerado en limón y ají), naranjas, limón, uvas, piñas, manzanas, membrillos, papayas, nísperos, chirimoya, sandía, melón, pepinos y todas las frutas cítricas.

CUADRO 9

Alergenos más frecuentes del asma bronquial en la Costa, Sierra y Selva del Perú.

	Alérgenos	Costa Porcentaje	Sierra Porcentaje	Selva Porcentaje
1.	Polvo de casa	92.86 %	2 %	8 %
2.	Lana de angora o cachemira	70 %		
3.	Papel viejo	56 %		12 %
4.	Relleno de colchón (lana vegetal, paja, crin, algodón)	51.99 %	7 %	17 %
5.	Materiales de construcción civil (cemento, cal, yeso)	48.9 %		3 %
6.	Hongos atmosféricos	47.22 %		11 %
7.	Pelos de auquénidos (llama, alpaca, vicuña)	44 %	25.4 %	
8.	Aserrín de madera	43.77 %	1 %	12 %
9.	Plumas de aves	41.6 %	3 %	9 %
10.	Detergentes	37 %		
11.	Tejidos (lana, algodón, chenille)	34.55 %	2 %	21 %
12.	Harina de pescado	32.3 %		
13.	Gérmenes	31.45 %		
14.	Alimentos	30.2 %	4 %	18 %
15.	Polvillo de cueros curtidos	26.8 %		
16.	Hongos patógenos	26.6 %		
17.	Harina de trigo	24 %		
18.	Polvillo de cereales y menestras.	18.4 %		
19.	Epitelios de animales	17.98 %		
20.	Tabaco	16.9 %		
21.	Insecticidas	16 %		Barbasco, 13 %
22.	Pelos de perro y gato	9 %	2 %	
23.	Hormonas	3.9 %		
24.	Polen	No hay cuadros típicos de polinosis; sólo agravan los síndromes respiratorios.		

CUADRO 10

Intradermorreacciones con alergenos inhalantes de la Costa practicadas en asmáticos de la Sierra.

Alérgenos	Asmáticos de la Costa	Asmáticos de la Sierra
1. Polvo de casa	+ + + + + +	+
2. Hongos atmosféricos	+ + +	— — —
3. Paja, crin	+ + +	— — — —
4. Polvillo de cereales y menestras	+ + + +	+
5. Pieles (llama, alpaca, vicuña)	+ + + +	+ +
6. Lana	+ + +	+ + +
7. Papel viejo	+ + + +	— — —
8. Materiales de construcción civil (cemento, cal, yeso)	+ + +	— — —

ETIOLOGIC FACTORS OF ASTHMA IN SWEDEN

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Three factors are of special importance concerning asthmatic patients (COLLDAHL 1956).

1. Is there any specific hypersensitivity in the bronchial mucosa that to a larger or lesser degree can provoke symptoms in the respiratory tract?
2. Is there any bronchial infection?
3. Is there any focal process in other parts of the body that in one way or another can irritate the bronchial process?

Of these factors the first can be considered as a primary cause, the second and the third are of great importance as they can precipitate attacks in patients where the real cause of asthma is not definitely known.

In Sweden a great interest has been displayed in the importance of specific hypersensitivity in asthma. The history of the patient and skin tests, together with skin titrations (testing starting with weaker solutions, which are gradually increased at 10 minutes intervals in order to determine the limit for positive skin reaction), are evaluated at the same time in order to determine if a positive skin test has any clinical importance.

Of eminent interest in the evaluation of the clinical importance of a positive skin test has been the comparison of this test with provocation test.

From a large body of work in this field the following may be mentioned.

SALÉN (1948) stressed that it is of great importance that the antigen analysis should be carried out with all such allergens which, having regard to the history and the answers to the questionnaire, and with due regard to occupation, habits and environment, might be supposed to be of causal significance to the patient concerned.

He made tests on a number of asthmatic children with extracts of dust from the homes of each of the children. A number of the children showed a strongly positive reaction to one dust extract which for a di-

fferent number of the children gave only relatively weak reactions, while strong reactions were obtained with one or more of the other dust extracts. From this emerges the necessity that the routine test should include not only one but several different dust extracts, selected in such a way that they cover so to speak the whole field of dust allergy. Storm van Leeuwen's important contributions to the elucidation of the causal signification of dust allergy to asthma and rhinitis cases in Europe must be given special mention in this connection. It is not infrequently an advantage to use what we call auto-dust, i.e. a dust extract from the patient's home. In case this procedure is not followed, we shall easily lose a number of cases of dust allergy, incomparably the most frequent form of allergy in Sweden.

SALÉN investigated 809 cases of allergy studied between 1941-46, including 794 cases of bronchial asthma. The material included 103 children under 12 years of age.

Of these 809 patients 618 had one or more positive skin reactions. Between 60-70 % of the skin positive cases reacted positively in the skin to one or more of the dust extracts used in the tests. The result of the skin tests on 36 asthmatic schoolchildren is seen in table 1.

TABLE 1

Intracutaneous skin test on 36 asthmatic schoolchildren. (Salén, 1948)

House dust		Mould		Horse		Dog		Cat	
Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
29	7	15	21	22	14	2	34	27	9
Cattle		Pollen		Fish		Milk		Egg	
Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
15	21	4	32	10	26	7	29	7	29

In studying asthma patients JUHLIN-DANNFELT (1950) made a provocation test for each of 165 strongly positive intradermal reactions. 99 of the provocation tests were positive and 66 negative. In most cases the test for hypersensitivity to animals and for alimentary allergy were carried out as exposure tests, whereas the tests for other inhalation allergens were performed as provocation tests, mostly by sniffing the substance in question. In the material of JUHLIN-DANNFELT the latent form of allergy of no actual clinical importance was no less than 2/5 of cases of hypersensitivity with pronounced skin reactions. The patients belonging to this material had in the main suffered from asthma throughout the year, and JUHLIN-DANNFELT stresses that it has usually not been practicable to decide in any other way than by means of exposure or provocation tests whether the demonstrable skin reactions were due to manifest or latent allergy. The positive reactions which were examined by

provocation tests in the material of JUHLIN-DANNFELT were to a large extent caused by animal epithelium and pollen (124 reactions). House dust was only examined in one case.

44 asthma patients were examined by COLLDAHL 1952 both by intradermal titration of in particular those antigens that had given a positive reaction in the intradermal routine skin test and by provocation tests. The outcome and number of these tests are found in table 2. In all 169 provocation tests were performed. On some patients only one provocation test was made, but on others up to nine provocation tests with different antigens were performed. Of the 169 provocation tests performed 57 were positive and 90 negative. It was possible by the method used, therefore, to get a clear result in about 87 % of the tests. In the remaining 13 % minor symptoms ocurred after inhalation of the strongest antigen solution (10^{-1}), but they were not sufficiently characteristic to indicate their being positive.

A very high incidence of positive results from the provocation tests has been obtained in cases of auto-dust allergy, no less than 64 % of the provocation tests with autodust being positive. Of the patients showing a positive skin reaction for auto-dust (by skin titration at least | for a concentration 10^{-3}) the provocation test turned out positively in 72 % for this antigen. The incidence of positive provocation tests of the whole material is about 34 %. Of the patients showing a positive skin reaction tests turned out positively for the whole material in 43 %.

As it is the opinion of several investigators that mould spores are the active agent in house dust, provocation tests with an extract of mixed mould spores * were performed particularly on dust-sensitive patients. As a rule a provocation test with mould spores was performed on such patients before the provocation test with auto-dust. As is seen from table 2, the tests with mould spores in the cases where the skin reaction was negative as a rule turned out negatively **.

Compared with the high percentage of positive provocation tests in cases with positive skin reactions to auto-dust it is interesting to find that only about 20 % of the provocation tests in cases of positive skin reactions to animal dander turned out positively. 58 % of the positive skin reactions to animal dander are of no clinical importance. In 22 % the result was questionable. Of the positive skin reactions for the whole material of asthma patients 42 % indicate with certainty a latent form of allergy with no actual clinical importance.

From this study it was also evident that the higher the skin sensitivity, the larger the percentage of positive results from the provocation tests (table 3).

As is seen from table 3 about 16 % of the provocation tests turn out positively in cases of a negative skin test (a one plus reaction at least does not occur after an intradermal injection of an antigen concentration $= 10^{-3}$) in asthmatic patients under the conditions for the mate-

(*) Containing Mucor, Penicillium, Botrytis and Hormodendrum.

(**) If the Skin test was positive to mould the provocation test turned out positively in 3 out of 7 tests.

rial in this investigation. Therefore, not a few patients with a clinically significant allergy may be missed if a negative skin test is considered as showing that a manifest form of allergy does not exist.

In case of positive skin reactions to different antigens, these reactions indicate a latent form of allergy of no clinical importance to a very different extent for different antigens. For autodust 14 %, for pollen 35 %, for mould 57 % and for animal dander 58 %. If by skin testing, therefore, one finds a positive reaction to animal dander, there is a much greater possibility that this skin allergy has no importance for the disease, than would be the case if one got a positive skin reaction for auto-dust.

It is very important to stress that the asthmatic troubles in a special case are often caused by multiple factors and that the result of the treatment depends upon how important factors could be more or less eliminated by the treatment.

As regards different asthmatic patients for whom specific hypersensitivity is of importance, the significance and importance of the specific factor for the development of the asthma can vary considerably.

In a series of 387 asthmatic patients investigated between 1950-53 at LUND, a small University town in the south of Sweden, by COLLDAHL 229 (59 per cent) showed a positive skin test while 158 (41 per cent) showed a negative. 43 per cent of a series of patients in this material with a positive intracutaneous test a positive provocation test.

Thus about 25 % of asthma patients could be shown to have a hypersensitivity of clinical importance.

FAGERBERG 1957 found the following figures for 400 asthma patients from Stockholm: 146 positive intracutaneous tests and 254 negative, eg. in 37 % the patients had a positive skin test.

TABLE 2
(Colldahl, 1952)

Antigen tested	Result of skin test	Result of provocation tests in per cent			Number of provocation tests performed
		+	?	—	
All antigens	+ and —	34	13	53	169
All antigens	+	43	15	42	111
All antigens	—	16	10	74	58
Autodust	+	72	14	14	29
Autodust	—	28	14	58	7
Pollen	+	59	6	35	17
Pollen	—	17	17	66	6
Moulds	+	43	0	57	7
Moulds	—	5	5	90	20
Animal epithelium	+	20	22	58	41
Animal epithelium	—	21	0	79	14
Cotton seed	+	50	17	33	6
Cotton seed	—	0	50	50	4

TABLE 3

(Colldahl, 1952)

Antigen concentration just giving a positive skin reaction by intracutaneous testing	Positive provocation tests in per cent	Number of performed provocation tests
10^{-5}	62	13
10^{-4}	45	33
10^{-3}	32	57
$> 10^{-3}$	16	58

KRAEPELIN and ENGSTRÖM (1956) investigated the frequency of manifest allergy in asthma patients in children.

In 119 patients up to 13 years of age 489 provocation tests were performed. Positive provocation tests were obtained in 65 %, but only stronger skin reactions were followed up by provocation tests. If all skin reactions, even the weaker ones, had been examined by provocation tests the percentage of positive provocation tests would have been less.

The authors could show just as has been shown 1952 for adult persons by COLLDAHL, that the percentage of positive provocation tests was larger if the skin reaction was stronger (see table 4).

TABLE 4

(Kraepelin and Engström, 1956)

Positive provocation tests/number of performed provocation tests

Skin reaction	+	+ +	+ + +	more than + + +
All allergens	9/51	78/159	128/174	103/105

The most important allergens were dust, animal dander, and pollen.

1957 KRAEPELIN and ENGSTRÖM reported on the results of specific hyposensitization in bronchial asthma in childhood. 110 patients were followed, 83 % of these were pure inhalation allergies, 2 % were pure food allergies and 15 % were combined food and inhalation allergies. The authors conclude that it appears that food allergies do not play an important part in childhood asthma in Sweden.

In a study of 748 adult asthmatic patients carried out in 1948, FAGERBERG (S;t Göran's Hospital, Stockholm) found that only about 18 % of the patients could be shown to have a manifest allergy. It was almost without exception inhalation allergens that caused the asthma; firstly dust, secondly pollen of different kinds and animal epithelials. It also could be shown that some patients were sensitive to more than one allergen.

FAGERBERG stressed the importance of carrying out a proper investigation. Out of the 748 patients accounted for here, not less than 280 had a total of 1107 positive i.c. reactions, but out of these, only 195 were shown to have clinical significance in a total of 133 patients. Thus 147 patients had 912 positive skin reactions without corresponding clinical manifestation. These figures give further support to the importance of not deciding on a specific hyposensitization with the help of only a skin test.

In this series therefore more than 80 % suffered from the type of asthma, the cause of which could not be attributed to any particular allergen.

FAGERBERG (1958) also examined the detailed circumstances connected with the onset of asthma in the S:t GÖRAN material consisting of 615 patients with endogenous asthma and 133 with exogenous asthma. The investigations have confirmed the discoveries of other authors that in a considerable percentage of cases infections can be considered as the real cause of the first attack of asthma in the patients. In the present material this occurred in 61,9 % of the patients with endogenous asthma and in 42,8 % of the patients with exogenous asthma.

The investigation has also shown that in the present material BSR cannot be used to differentiate between asthma with and without infections in the air passages, lungs or sinus. An increased BSR, however, renders such an infection more likely than a normal BSR.

ARNER, FÜRST and TRUEDSSON (1958) found in a study of 378 asthma patients examined at the Medicine Clinic at LUND that in 46 % the patients had one or more positive reactions in the skin test. Positive skin tests were found in 2/3 of the patients up to the age of 30, in 1/2 of the patients between 30-39 years of age and in 1/4 of the patients between 40-59.

The antigens that most often gave a positive skin reaction were dust, animal dander and pollen.

In 131 asthma patients between 2-69 years of age who had one or more positive skin reactions a positive provocation test was found in 75 %. Therefore a positive skin reaction of etiologic importance in this series of asthma patients was found in 3/4 of 46 % that is 34,5 %.

RIPE (1962) has recently studied the importance of mould allergy in Sweden with specially prepared mould extracts (PALMSTIERNA and RIPE). He has found that mould allergy has a clinical importance in about 12 % of patients investigated. These figures are larger than in earlier investigations. Among these the following may be mentioned.

NILSBY (Örebro, Sweden) found in a group of 123 asthma patients 12 patients (10 %) where anamnestic data and the result of the intra-

cutaneous test with mould extracts spoke for a sensitivity of causal significance.

In 1952 COLLDAHL found (table 2) that if the skin test was positive al least in a concentration 1:1 000, the provocation test turned out positively in about 40 %. If the skin test was negative in a concentration 1:1 000, the provocation test was positive in not more than 10 %.

FAGERBERG (Uppsala, Sweden) performed provocation tests with mould extract on 76 patients and could establish that 25 (33 %) reacted positively on the provocation test in spite of the fact that many of them (20) had not reacted positively to the intracutaneous test (1:1 000 weight/volume).

PALMSTIERNA and RIPE have modified the apparatus of Pirt from Porton laboratories in England for cultivation of microorganisms and adapted it for cultivation of allergenic moulds. The mould is cultivated submerged at a constant temperature, at constant pH, under constant aeration in a medium consisting of casein hydrolyzate, glucose and salts. When the mould has grown out maximally it is harvested, disintegrated and extracted by centrifugation at 50 000 x g for 120 minutes at 0° C. The extracts are diluted following the nitrogen values where 1:10 = 1 mg N/ml. This corresponds approximately to 1:10 dry weight/volume.

RIPE is just now occupied in investigating the incidence of mould allergy in Stockholm, Sweden, by intracutaneous and provocation tests with extract produced in the above mentioned way. He is using eight different moulds, which have been proven to be common in the Stockholm air. Botrytis cinerea, two Penicillium species, Cladosporium herbarum, Pullularia pullulans, Alternaria tenuis, Mucor hiemalis and Aspergillus niger. The preliminary results appears in tables (5 and 6). The patients are principally composed of asthma and rhinopathia. Among the patients are a considerable number whose symptoms are of very long duration and many of them are treated with steroids.

Most of the patients react to several mould species, as well as to several allergens such as pollen and dust.

It is not clear how often food allergy is of importance as regards asthma. According to the investigations mentioned earlier the incidence of food allergy of importance as regards asthma is not large, though there are, however, reasons to believe that this form of allergy has a greater bearing on asthma than it has hitherto been possible to verify.

In an investigation not yet published (COLLDAHL) it could be shown that allergic symptoms can disappear if the diet is profoundly changed, e. g. by giving the patient the elimination diets prescribed by Rowe.

BRONCHIAL INFECTION.

Bronchial infection is an important factor in provoking asthma in asthmatic patients. Investigations performed with a special method for obtaining bronchial secretion in a sterile way have shown (BERGMAN, COLLDAHL and NILSSON, 1954) that at least 90 % of the patients with

TABLE 5

Intracutaneous test with mould extracts (Ripe)

Age	Diagnos.	Number of cases	Number of positive intracutaneous tests with the following concentrations (1:10 = 1 mg N/ml)					Totally
			1:300	1:1000	1:10000	1:100000	1:1 milj.	
16-68	Asthma bronchiale and Rhinitis allergica.	550	65 (12 %)	40 (7 %)	21 (4 %)	9 (2 %)	1 (0,2 %)	136 (25 %)

After a positive reaction no stronger solutions was used.

TABLE 6

Result of provocation tests by inhalation or by dripping into the nostrils with the following concentration (1:10 = 1 mg N/ml). (Ripe)

	1:300		1:1000		1:10000		1:100000		Totally	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
Number of provocation test.	24 (50 %)	25 (50 %)	24 (66 %)	12 (34 %)	12 (80 %)	3 (20 %)	3 (100 %)	0	63 (60 %)	40 (40 %)

When a positive reaction is obtained no stronger solutions was used.

healthy lungs were sterile in the lower part of the trachea and in the larger bronchi.

Asthmatic patients with no apparent increase of BSR were not found to have bronchial infection more frequently than persons with healthy lungs. Comparison with the two groups mentioned earlier shows that asthmatic patients with enhanced BSR had bronchial infection more often than those.

In addition to the direct culture-response, differential counts made from the bronchial secretion collected by sterile suction according to the given method indicate whether an infection exists, and in that case the neutrophilic leucocytes are increased. An almost exclusive eosinophilic finding argues against infection.

A study has been performed (COLLDAHL, 1960) of the presence of neutrophil and eosinophil cells in the sputum—principally in cases of asthma, but also of other diseases of the lungs and bronchial tract. In conjunction with the erythrocyte sedimentation rate the cell distribution in the sputum can provide information of value in diagnosis and therapy. This might well be used as a routine measure in the investigation of lung diseases.

Both neutrophil and eosinophil leukocytes were found in the washed sputum from the asthma patients. In cases of asthma without coincident diseases the erythrocyte sedimentation rate was normal, or only slightly elevated.

In most of the cases of asthma accompanied by other diseases the ESR was elevated. Such conditions included sinusitis, bronchitis, bronchopneumonia, cholecystitis and cardiac infarction. Both neutrophil and eosinophil leukocytes were present in the sputum, the former occasionally predominating.

Eosinophils occurred in the sputum in a number of other diseases than asthma and allergic rhinopathy. The most marked preponderance of eosinophil cells was found in cases of Löffler's syndrome. The erytrocyte sedimentation rate was often quite high.

SUMMARY.

According to the investigations mentioned in about 50 % of asthmatic patients a positive result is obtained by skin testing with one or more antigens. If these antigens are tested by provocation a positive result is obtained in about 50 % of the patients. That means that 1/4 of the asthma patients are sensitive to an antigen which is of importance for their asthma.

The most important antigens are the following: dust, pollen, moulds, animal dander. According to the investigations mentioned earlier the incidence of food allergy of importance as regards asthma is not large, though there are, however, reasons to believe that this form of allergy has a greater bearing on asthma than it has hitherto been possible to verify.

A small number of patients are sensitive to an antigen even if the skin test is negative. But in about 60-70 % of the asthmatic patients we cannot find an antigen which can provoke symptoms by provocation tests.

In about 1/3 of asthma patients admitted to hospital for asthma a bronchial infection is found that could have praecipitated the asthmatic attack.

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FRECUENCIA ETIOLOGICA DEL ASMA BRONQUIAL EN MEXICO

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I.—PREÁMBULO.

El asmático, en la República Mexicana, etiológicamente es polifacético; en general, puede compararse a un diapasón que vibra idénticamente con excitantes diversos. Son contados los enfermos en los que hay sólo un factor como causa de su padecimiento.

La etiología varía en forma importante, de acuerdo con la edad y el sexo. Es interesante señalar que los paroxismos asmáticos sólo ocasionalmente se han encontrado presentes en los primeros meses de la vida, pero desde la edad preescolar aumentan y su frecuencia es mayor en la tercera o cuarta década. Por lo que se refiere al sexo, predomina en el masculino, observándose en particular en trabajadores especializados, como los de fábricas de hilados y tejidos, ingenios azucareros, pizcadores, despepitadores de algodón y algunos otros; es de señalarse que en la República Mexicana la enorme mayoría de obreros pertenece al sexo masculino.

El pronóstico, íntimamente relacionado con la etiología, es más o menos benigno si predominan el factor alérgico o el endocrino; si es el infeccioso, psicológico o intrínseco, es de relativa severidad.

Alrededor de las costas, que tienen una longitud de 9.903 km. (7.147 corresponden al Pacífico y 2.756 al Golfo), existen las llamadas *zonas asmógenas,* importantes no sólo por el extraordinario número de pacientes asmáticos encontrados, por la intensidad, severidad y duración de los paroxismos, sino también por la existencia de *enfermos barómetros,* llamados así porque anuncian, por medio de la aparición de sus síntomas, hasta con varias horas de anticipación, la iniciación de perturbaciones climatológicas.

II.—CARACTERÍSTICAS GEOGRÁFICAS Y METEOROLÓGICAS DE LA REPÚBLICA MEXICANA.

Situación.—México forma parte del continente americano y se halla dentro del hemisferio norte. Su extensión es de 1.967.183 km^2.

Las coordenadas geográficas de sus puntos extremos corresponden: la más boreal, a los 32° 43' latitud norte, en la frontera con los Estados

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Unidos, y la más meridional, a la desembocadura del río Suchiate, en la frontera con Guatemala, a los 14° 30' latitud norte. El punto más oriental está situado en el litoral noreste de la Península de Yucatán, en el Mar de las Antillas, a los 86° 46' longitud oeste de Greenwich, y el más occidental está en el litoral del Océano Pacífico, al noreste de la Península de Baja California, a los 117° 08' longitud oeste Greenwich.

La República Mexicana colinda al norte con los Estados Unidos de América y al sureste con Guatemala y Honduras Británicas, ubicándose en dos grandes porciones de nuestro continente: América del Norte y América Central.

Fisiografía.—Es muy accidentada, pudiendo considerarse una meseta central limitada por las siguientes cadenas montañosas: Sierra Madre Oriental, Sierra Madre Occidental y Sierra Volcánica Transversal; las dos primeras cruzan la República de norte a sur, convergiendo hacia la última, constituyendo el Nudo del Cempoaltépetl, a la altura del paralelo de los 19° y que es tanto un límite altimétrico como geológico, climatológico y biogeográfico. Ahí se encuentran las cumbres más elevadas, como el Citlaltépetl (5.700 m.), Popocatépetl (5.452 m.) e Iztaccíhuatl (5.286 m.).

En la altiplanicie central está la Ciudad de México, a los 19° 26' latitud norte y a los 2.240 m. de altura, asiento del núcleo humano alrededor del cual se ha integrado y desarrollado la nación, política, cultural y económicamente.

Zonas Climatológicas.—El Trópico de Cáncer cruza el territorio mexicano hacia su parte media, estableciendo dos zonas climatológicas casi iguales en extensión, una templada al norte y otra tropical al sur, pero debido a la gran altura a que se encuentra la mayor parte de su territorio, pues sólo el 32 por 100 se sitúa a menos de 500 metros de elevación y el 68 por 100 restante va de los 501 a más de 2.500 metros de altura sobre el nivel del mar, se producen modificaciones en el clima de la República y sobre todo en su régimen térmico, como consecuencia de la predominancia de la altura sobre la latitud; por ello se encuentran (clasificación de Köepen) climas tan variados como los lluviosos tropicales (de sabana, bosque tropical, selva), secos (estepa, desierto) y lluviosos templados (seco en invierno, húmedo, seco en verano, con lluvias raras, pero intensas en todas las estaciones).

En cuanto a la repartición de las lluvias en el territorio mexicano, se determinan las zonas siguientes: húmeda, que comprende el 6,8 por 100; semi-húmeda, con el 10,5 por 100, y semiárida y árida, el 82,7 por 100 de la superficie del país.

Nublados.—Es posible considerar cuatro grandes zonas: la parte norte de Baja California con un total de treinta días nublados al año; la meseta central con sesenta; parte noroeste, centro, oeste y zona sur con noventa, y el sureste, sobre todo en Chiapas, con ciento cincuenta días.

III.—Principales factores etiológicos del asma bronquial.

Datos estadísticos basados en la experiencia de médicos que residen en distintas zonas de la República Mexicana, han señalado que en términos generales los factores etiológicos en el asma bronquial varían en forma muy importante con la edad, las características climatológicas, los factores económico-sociales, la alimentación, etc., pero puede mencionarse que los más importantes son:

A) Alérgicos.
B) Infecciosos.
C) Psicológicos.
D) Endocrinos.
E) Intrínsecos.

A) *Alérgicos.*

En la mayoría de los enfermos existen polisensibilizaciones. Entre los alergenos que más frecuentemente se han encontrado como productores de síntomas están:

ALERGENOS FRECUENTES EN EL ASMA DEL MEXICANO

1) INHALANTES .....
   - *a)* Polvo casero.
   - *b)* Epitelio de animales.
   - *c)* Hongos atmosféricos.
   - *d)* Pólenes.
   - *e)* Olores diversos.
   - *f)* Fibras vegetales.
   - *g)* Humo y productos de combustión.

2) INGESTANTES .....
   - *a)* Alimentos.
   - *b)* Productos químicos.

3) ENDOPARASITOS

4) ECTOPARASITOS E INSECTICIDAS

5) OCUPACIONALES

6) REGIONALES

1) *Alergenos inhalantes.*—Indiscutiblemente, los más importantes. Entre ellos:

a) Polvo casero.

Clínicamente ofensor en la casi totalidad de los pacientes, su contenido inmunológico está en relación con las características ambientales del sitio donde se colectó, variando notablemente en las distintas zonas de la República Mexicana (citadinas, agrícolas, ganaderas, industriales, etcétera).

Es característica en la Ciudad de México la presencia en la atmósfera de grandes tolvaneras durante los meses de febrero y marzo, época en que los enfermos asmáticos exacerban o desencadenan con más frecuencia sus síntomas.

Debe mencionarse que se ha encontrado que es todavía más potente el polvo envejecido, posiblemente porque sus componentes se encuentran en mayor desintegración y por la asociación con parásitos, ácaros, hongos y otras substancias.

En las zonas asmógenas, es notoria la gran potencia ofensora del polvo que se encuentra en cuartos que han permanecido cerrados por un período de tiempo más o menos largo.

b) Epitelios de animales.

Son alergenos muy importantes, llegando a considerarse, después del polvo, el inhalante no estacional más común.

Son de mencionarse plumas, lana, pelos (de gato, perro, caballo, conejo, ganado, etc.), tanto en zonas no urbanas por existencia de los animales, como en las ciudades, porque muchos de los objetos personales o de casa están hechos con ellos.

c) Hongos atmosféricos.

Aun cuando presentes en todo el país, se observa que son de extraordinaria importancia en las zonas asmógenas.

Variando la cantidad y la especie, de acuerdo con la situación geográfica y climatológica, los más frecuentes en la ciudad de México son: Alternaria, Hormodendrum y Helminthosporium. Estudios realizados dentro de las casas habitación señalaron a Monilia, Penicillium, Hormodendrum y Alternaria.

En la ciudad de Monterrey (al norte del país), Alternaria, Hormodendrum, Penicillium y Aspergillus.

En Jalapa, Veracruz, en orden decreciente, Hormodendrum, Penicillium, Rhizopus, Streptomyces, Cephalosporium, Helminthosporium, Alternaria, Absidia y Mucor.

d) Pólenes.

Es posible afirmar que no son un alergeno de gran importancia en México, debiendo recalcarse también que la mayoría de los pacientes son alérgicos a varios pólenes. En el territorio norte se presentan enfermos con sensibilizaciones a uno o dos pólenes.

En la ciudad de México se han observado los siguientes períodos de polinación:

Arboles, de noviembre a abril, siendo más importante el Fraxinus.

Pastos, de mayo a octubre, con máximas cuentas en mayo y septiembre, siendo más importante el Cynodon Dactylon.

Los *zacates* no son muy ofensores; el índice de la Ambrosía es cuatro para México y Tampico.

En el resto de la República, en forma general, puede señalarse la siguiente división polínica:

Zona central, con datos similares a los de la Ciudad de México.

Zona noroeste, con bastantes zacates y pastos (Amaranthus hibridus y retroflexus, Franseria tenuifolia, Ambrosia psylostachia, Holcus halepensis y Cynodon Dactylon.

Zona norte, que tiene también una rica flora alergénica (Holcus halepensis, Cynodon dactylon, Phelum pratense, Amaranthaceas, Quenopodiaceas, Franseria tenuifolia, Parthenium, Ligustrum y Prosopis).

Zona sudeste, en la cual aparentemente no existen pólenes alergénicos en cantidad apreciable.

e) Olores diversos.

Entre ellos deben mencionarse de productos de belleza y pinturas.

De productos de belleza:

Constantemente se observan como desencadenantes de síntomas en las personas que los usan o en las que están expuestas a su contacto.

Por su frecuencia, son de interés los perfumes, las lacas nebulizadas para el cabello, desodorantes y maquillaje.

De pinturas y sus componentes:

Paroxismos asmáticos extraordinariamente molestos han sido observados con ellas. También síntomas, aunque no muy severos, en obreros que fabrican o manejan las pinturas o sus componentes, como la gasolina, thinner y aguarrás.

f) Fibras vegetales.

Interesantes, ya que muchas veces el paciente ignora que las inhala porque éstas se encuentran formando parte del relleno de colchones, almohadas, muebles, etc., o integrando diversos objetos de ornato o de uso diario.

Entre las más comunes se encuentran algodón, pochote, yute, lino, henequén.

g) Humo y productos de combustión.

Muy importantes en ciudades industriales, ya que se encuentran difundidos en forma extraordinariamente amplia y constante en la atmósfera.

En la época actual puede decirse, por un lado, que son raras las personas que no fuman o no están cerca de quienes lo hacen, y por otro, con el número creciente de industrias y de vehículos de transporte que tienen que verter humos y productos de combustión a la atmósfera, es casi imposible evitarlos.

2) *Alergenos ingestantes.*

Entre ellos es posible considerar los alimentos y productos químicos.

De los alimentos, aun cuando varían en las distintas zonas de la Re-

pública, los más importantes son: chocolate, nuez, pistache, piñón, avellana, chícharo, huevo, lentejas, mangos, mariscos, pescados, maíz, etc.

De los productos químicos, que cada día aumentan en importancia como factores etiológicos del asma, es posible mencionar: antibióticos, bacteriostáticos, aspirina.

3) *Endoparásitos.*

Son de extraordinaria importancia en los asmáticos, endoparasitosis diversas, particularmente en aparato digestivo.

4) *Ectoparásitos e insecticidas.*

Se han encontrado casos en que los ectoparásitos han desencadenado paroxismos severos de asma, particularmente avispas, chinches, pulgas y mosquitos.

Los repelentes e insecticidas con bastante frecuencia producen molestias, sobre todo los hechos con piretro y D. D. T.

5) *Alergenos ocupacionales.*

Cada vez con más frecuencia se encuentra el factor ocupacional como desencadenante de asma, particularmente en las fábricas que manejan lana, algodón, algunos alimentos. Las leyes mexicanas no consideran este padecimiento ocupacional como enfermedad profesional.

6) *Alergenos regionales.*

Existen zonas asmógenas particularmente alrededor de las costas y en las zonas agrícolas y algodoneras.

B) *Alergenos infecciosos.*

Este factor es de extraordinaria importancia, y su presencia ha sido observada en todas las edades, desencadenando, manteniendo o exacerbando en forma severa el padecimiento. Es casi seguro encontrarlo en los asmas crónicos y en los rebeldes al tratamiento habitual. Puede actuar como:

1.° Focos infecciosos en amígdalas, adenoides, dientes, senos paranasales, vesícula biliar, próstata, órganos genitales femeninos, etc.

2.° Procesos infecciosos generales desencadenados por gérmenes, virus, hongos.

Se están realizando estudios estadísticos en poblaciones tipo de distintas zonas de la República Mexicana, con el objeto de conocer los gérmenes que se encuentran en la secreción bronquial de los asmáticos en diferentes fases del paroxismo.

C) *Psicológicos.*

De extraordinaria importancia, creciente cada vez más, este factor se ha encontrado presente tanto en el paciente como en el medio ambiente; varía de acuerdo con edad, sexo, situación económica, estado civil, ocupación, etc.

En el medio ambiente motiva diferentes aspectos en la escuela, el trabajo, el hogar.

Sensación de culpabilidad, deseos de evitar castigos, frustraciones, buen o mal ajuste familiar, interrelaciones normales o anormales con sus semejantes, etc., llegan a crear un círculo vicioso que repercute en forma totalmente negativa en la evolución del padecimiento.

D) *Endocrinos.*

Disendocrinias de cualquier tipo han demostrado su posibilidad como factores etiológicos importantes en los asmáticos. Particularmente interesantes son las de las glándulas genitales, tiroides, etc.; pubertad, menstruación y climaterio deben siempre tomarse en consideración.

E) *Intrínsecos.*

Aun cuando afortunadamente es pequeño el grupo de asmáticos en los cuales es imposible descubrir su o sus agentes etiológicos, dada la severidad y constancia de los síntomas que presentan, su importancia clínica debe ser tomada en consideración.

Sumario.

México, situado en el hemisferio norte del Continente Americano, con extensión de 1.967.183 km^2, presenta características climatológicas y orográficas que determinan aspectos especiales en los factores etiológicos del asma bronquial; estadísticas realizadas señalan que los más importantes son: los *alérgicos, infecciosos, psicológicos, endocrinos* o *intrínsecos.*

Como alergenos característicos pueden señalarse: En la Ciudad de México, el polvo de las tolvaneras, presentes en su atmósfera durante los meses de febrero y marzo; en la República Mexicana, en general, los de las zonas asmógenas, localizadas en las regiones agrícolas, algodoneras y alrededor de las costas; en estas últimas con frecuencia se observan los enfermos barómetros, que anuncian, con la aparición de sus síntomas, hasta varias horas antes, la iniciación de perturbaciones climatológicas.

Los pólenes, en general, no son alergenos de gran interés, siendo rarísimos los pacientes sensibles a uno solo; en el territorio norte sí se observan sensibilizaciones a uno o dos pólenes.

Los hongos atmosféricos cada día son de más importancia, particularmente en las zonas asmógenas o en los cuartos cerrados durante bastante tiempo.

De las fibras vegetales como alergeno regional se puede mencionar al henequén en Yucatán (Sureste).

Los perfumes, las lacas nebulizadas, desodorantes, así como pinturas y sus componentes (gasolina, thinner y aguarrás) son factores etiológicos interesantes.

México se industrializa con rapidez y el humo y los productos de combustión (fábricas, vehículos de transporte) se mencionan cada vez con más frecuencia; lo mismo sucede con los alergenos ocupacionales observados en obreros que prestan sus servicios en fábricas de hilados y tejidos, colectadores de algodón, despepitadores, ingenios cañeros. Esto también permite señalar que en el sexo masculino la incidencia de asma es mayor, ya que a estos factores etiológicos la mujer, en su gran mayoría, todavía no está expuesta.

Endoparásitos y ectoparásitos también se han encontrado frecuentemente como causantes de asma.

Los factores infecciosos, psicológicos, endocrinos e intrínsecos, aun cuando son de gran importancia, no ofrecen características especiales entre los asmáticos mexicanos.

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FRECUENCIA DE LOS FACTORES ETIOLOGICOS DEL ASMA EN PORTUGAL

M. DAMAS MORA. (*)

Las diversas investigaciones realizadas por la Sociedad Portuguesa de Alergia durante los años de 1957 a 1962, nos permiten, a través de las informaciones que nos han sido comunicadas por los miembros interesados dispersos en varias zonas del país, hacernos una idea aproximada de la frecuenca de los factores etiológicos del asma en Portugal.

Esos trabajos están basados en la observación de algunos millares de enfermos de diversas edades, estados y profesiones y, aunque no nos permitan afirmar exactamente cuál es el verdadero factor etiológico desencadenante de la crisis, nos permiten apreciar en forma conjunta el estado actual del problema.

Ya anteriormente, en un trabajo presentado a la II Conferencia Internacional de Asmología, tuve la oportunidad de abordar aquel problema desde un punto de vista estadístico, lo cual permite darnos cuenta de la gravedad de esta enfermedad entre nosotros, pudiéndose deducir, en consecuencia, que existe un porcentaje de asmáticos de alrededor del 1 por 100 de la población válida.

La diversidad de subclimas que pueden señalarse en las varias regiones del país—resultado de su configuración geográfica a lo largo del Océano Atlántico, en toda la extensión de la costa—se traduce en alteraciones climáticas, a las que cabe atribuir, muchas veces, la aparición de las crisis asmáticas, lo cual tiene aspectos de una cierta importancia.

Sin embargo, dentro de los subclimas, los microclimas —noción expuesta por primera vez en 1931 por los científicos alemanes—, son los que presentan variedades más dispares. Esos microclimas que, al decir del fallecido investigador luso, Doctor ARRUDA FURTADO "pueden no destacarse por su extensión pero sí, ciertamente, por la diferencia intensa de unos a otros, influyen, además, por sus características coadyuvantes, en las crisis de asma y, también, paradójicamente, en ciertos casos, en su terapéutica, por las indicaciones perentorias de un cambio de clima para su cura".

(*) Secretario General de la Sociedad Portuguesa de Alergia, Vicepresidente de la International Association of Asthmologists (INTERASMA).

En octubre de 1957, afirmábamos la influencia del clima en el origen de los factores desencadenantes de los diversos tipos de asma, aunque tuviéramos, como es obvio, que relacionarlos con la etiopatogenia de la enfermedad, de acuerdo con el criterio adoptado entonces por nosotros.

Más tarde, en el trabajo presentado en Barcelona al V Curso de Enfermedades Alérgicas realizado en la Facultad de Medicina, en la Cátedra de Patología General del Profesor FERNÁNDEZ CRUZ, bajo la orientación del ilustre especialista Doctor FARRERONS-CO, tuvimos la oportunidad de exponer nuestro punto de vista sobre el carácter extremadamente multiforme de la etiología del asma bronquial.

Para nosotros, de acuerdo en gran parte con el concepto de FROUTCHMAN y otros autores, hay que encararlo bajo el aspecto clínico, en relación con los caracteres etiopatogénicos, lo cual, nos llevó a admitir dos formas esenciales de asma:

1.ª El asma bronquial primario —producido originariamente por una hipersensibilidad— (el asma, en este caso, es una enfermedad exclusivamente provocada por factores específicos (alergenos) o no específicos (patergenos) como el calor, el frío, el viento, los gases, los factores emocionales, los esfuerzos físicos, etc.).

2.ª Los asmas secundarios, de reacciones condicionadas por otros procesos de localización y naturaleza diversa, en ocasiones, de mayor importancia que el factor inicial.

De las citadas investigaciones, podemos deducir, según los informes que nos han sido comunicados, que la frecuencia de los factores etiológicos del asma en Portugal, se sitúan:

a) En las variaciones climáticas de los subclimas y, dentro de éstos, de los microclimas (ARRUDA FURTADO, DAMAS MORA).

b) En la polinización de la atmósfera por las especies frecuentes y comunes entre nosotros: *Cupressus Sempervirens, Pinus, Parietaria Officinalis, Gramineas, Olea Europea, Quenopodiáceas y Amarantaceas, Xanthium* y *Artemisia, Plantago* (QUITERIA PINTO DA SILVA).

La fuerte reacción positiva de los "tests" observados en diversos grupos de individuos en tratamiento, se refieren, en su mayoría, a los pólenes de las Dactilis, Hordeum Festuca, Secale cereale, Polypogon, Zea Mays (PIEDADE GUERREIRO, DIAS DE ANDRADE).

c) En la sensibilización al polvo de las casas y de los locales de trabajo: a los cereales, y a los hongos solos o asociados a sustancias bacterianas (CORREIA GUIMARAES, LOPES VASQUES).

d) En la existencia de múltiples afecciones crónicas del aparato respiratorio, como las bronquitis, las bronquiectásias, las neoplasias, las pneumoconiosis y otras (LOPO DE CARVALHO, PALMA CARLOS).

e) En la gran frecuencia de las insuficiencias cardíacas, mitrales e hipertensas (ADELINO PADESCA).

f) En los desarreglos y pertubaciones alimenticias (CORREIA GUIMARAES).

g) En las alteraciones tóxicoinfecciosas y agudas relacionadas con la tuberculosis y la gripe (DAMAS MORA).

SUMMARY

The author mentioning received informations concerning the frequency of etiological factors of Asthma in Portugal imputes them to:

a) The climateric variations of subclimates and within them of microclimates (ARRUDA FURTADO, DAMAS MORA).

b) The atmosphere pollination by the ordinary and common species among us: *Cupressus Sempervirens, Pinus, Parietaria Officinalis, Gramineas, Olea Europea, Quenopodeaceas* and *Amarantaccas, Xanthium* and *Artemisia, Plantago* (QUITÉRIA PINTO DA SILVA).

The strong positivity of "tests" observed in several groups of individuals under treatment is reported in the majority to the pollens of Dactilis, Hordeum, Festuca, Secale cereale, Polypogon, Zea Mays (PIEDADE GUERREIRO, DIAS DE ANDRADE).

c) The sensitization to dust of home and work places: to all sorts of grain and fungus alone or associated to bacterial substances (CORREIA GUIMARAES, LOPES VASQUES).

d) The existence of multiple chronical affections of respiratory apparatus such as bronchitis, bronchiectasis, neoplasis, pneumoconiosis and others (LOPO DE CARVALHO, PALMA CARLOS).

e) The great frequency of cardial insuficiencies mitrals and hypertenses (ADELINO PADESCA).

f) Nourishing unrulinesses and perturbations (CORREIA GUIMARAES.)

g) Acute and toxi-infectious perturbations connected with tuberculosis and influenza (DAMAS MORA).

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THE FREQUENCY OF AETIOLOGIC FACTORS IN BRONCHIAL ASTHMA IN FINLAND

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The frequency of allergic diseases in Finland is in accord with the number given in most countries. But Finland's geographical position and its climate as the most northern country in the world besides Iceland greatly contribute to the causes of allergic diseases. The lovely summers with their long, light nights fly too fast. The three very much awaited sunny months which, unfortunately, can also be rainy and cold cannot give the inhabitants the strength to bear the burden of the long, dark and severe winter months. For this reason bronchial asthma characterized by infections of shorter or longer duration is the most common allergic disease. When it is dark and cold one wishes to stay indoors and so one suffers from lack of fresh air. Nutriment becomes monotonous and lacks vitamins and imported foodstuff is expensive. The numerous airtight clothes necessary in wintertime promote the occurrence of allergic eczema which after asthma is the most common form of allergy.

Chronic infections in different parts of the body, mainly in the upper and lower respiratory tract, are the most common causes of bronchial asthma in adults and also in children from 7 years on. Of 500 asthmatic schoolchildren treated in the wards of the Allergy Hospital in Helsinki, 88 % showed chronic infections, mostly in the nasal sinuses, adenoids, tonsils or teeth. In only 12 % no focal infection could be found. 200 adult asthmatics were x-rayed and examined by the hospital's otolaryngologist and dentist. 18,5 % had nasal polyps, sinuitis was found in 72 % of which 21,5 % had pansinuitis, 20 % chronic tonsillitis and 58 % periapical infections of the teeth. In only 10 % of the patients studied no signs of chronic infections could be found. In the infected cases it was established that no definite and lasting improvement of the asthma could be seen before sanitation of the foci of infection was performed.

Extrinsic factors such as inhalants are also of importance. In the above mentioned material 36 % of the asthmatic patients showed marked positive skintests to different allergens other than bacteria. In adults housedust is the most common sensitizer found. But in our agricultural country allergies to animal emanation are also often seen. Sensitivity

to cowsshair, milk and beef can be found in the same patient. Homeknitted woolen stockings are still used in wintertime and also hand-knitted or woven clothes. Woolen blankets and rugs cover beds, floors and walls and often induce sensitivity. Poultry farmers are sensitive to feathers which also are still frequently used in pillows and mattresses.

Food allergy is mostly seen in infants and small children, but also occasionally cause asthma in adults. Sensitivity to milk, wheat, eggs and chocolate are most common. In our country which boasts of 60.000 lakes fish allergy is often seen and much feared because of the nutritional difficulty it brings forth.

Sensitivity to drugs, paint, detergents, cosmetics and all kinds of new industrial materials increases each year.

During last years fungi have been found in the sputa of some asthmatics, among which were a few children. Fungicidal treatment gave good results.

Slides show the frequency of the aetiologic factors mentioned in asthmatic children and adults.

HÄUFIGKEIT ÄTIOLOGISCHER FAKTOREN BEIM BRONCHIAL-ASTHMA IN DEUTSCHLAND

E. FUCHS (*)

Aus dem Allergie-Forschungs-Institut und der Asthma-Kilink, Bad Lippspringe/Westfalen, Deutschland (Chefarzt: Dr. med. W. Gronemeyer).

Die mir gestellte Aufgabe, über die Häufigkeit ätiologischer Faktoren beim Bronchialasthma in der Bundesrepublik Deutschland zu berichten, kann bisher aus vielen Gründen durch eine Sammelstatistik aus verschiedenen Kliniken und grossen Krankenhäusern oder Asthmazentren kaum befriedigend gelöst werden. Bei Erkrankungen, die einer Meldepflicht unterliegen, wie Infektionskrankheiten, Berufskrankheiten, beispielsweise im Sinne der in der Bundesrepublik Deutschland bestehenden Berufskrankheitenverordnung, ist es verhältnismässig einfach, einen zahlenmässigen Überblick, auch über ätiologische Faktoren, zu gewinnen. Der Anregung, entsprechende Statistiken und Untersuchungen aus verschiedenen Kliniken des Landes zusammenzustellen, um auf diese Weise einen Überblick zu erhalten, sind wir nicht gefolgt, wofür u. a. folgende Gründe zu nennen sind:

1. Die Diskussion über das Wesen des Asthma bronchiale, das weder ätiologisch noch klinisch ein einheitliches Syndrom darstellt, ist gerade bei uns in Deutschland in den letzten Jahren besonders lebhaft und keineswegs abgeschlossen, so dass auch schon aus diesem Grunde eine Art Sammelstatistik aus verschiedenen Kliniken ein falsches Bild ergeben würde.
2. Nur an einigen wenigen Kliniken werden in Deutschland ätiologische Analysen durchgeführt.
3. Bestehen zum jetzigen Zeitpunkt keine einheitlichen und gemeinsamen Kriterien bei der Untersuchung von Asthmatikern.

Wir hielten es daher für gerechtfertigt, ein grösseres Kollektiv des eigenen Krankengutes entsprechend statistisch zu erfassen und mit Hilfe von Lochkarten und elektronischen Maschinen (IBM) auszuwerten. Dabei erweist sich als ein besonderer Vorteil, dass sämtliche Asthmatiker bei uns in einem einheitlichen Klinikmilieu in gleicher Weise und nach einem gleichen Plan untersucht und "beurteilt", systematisiert und behandelt wurden. Da unser Einzugsgebiet Nord-, Süd und West-

(*) Unter Mitarbeit von cand. med. Uwe Gronemeyer und Fräulein Gisela Eberhardt.

deutschland umfasst, ist das Krankengut "repräsentativ" für die gesamte Bundesrepublik Deutschland, so dass im Hinblick auf die gestellten Fragen einige Aussagen möglich werden.

Unser nicht ausgewähltes Krankengut umfasst 3.000 Asthmatiker, die im Jahre 1963 im Allergie-Forschungs-Institut und der Asthma-

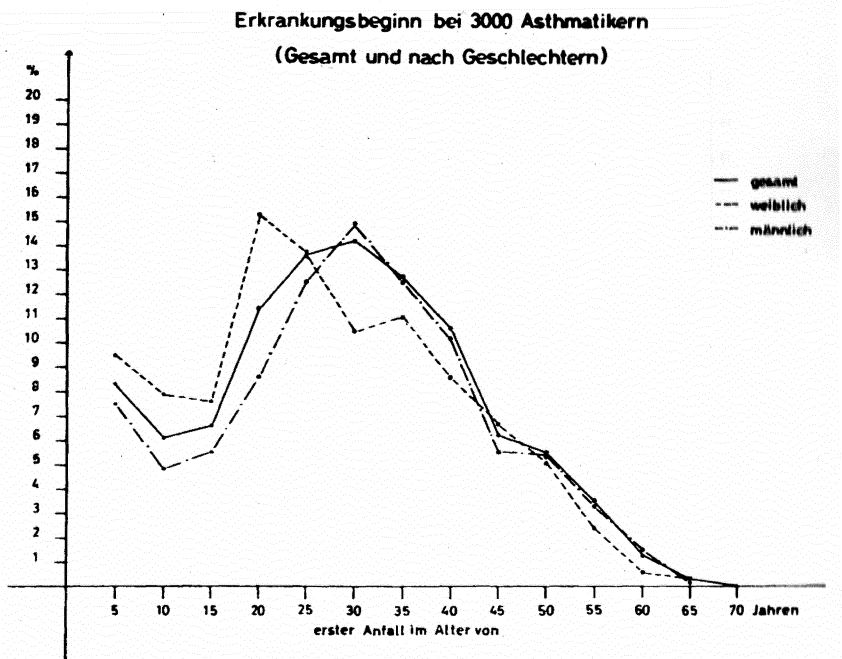

Abb. 1

Klinik, Bad Lippspringe untersucht und behandelt wurden. Bei der statistischen Erfassung haben wir

1. subjektive Angaben der Patienten (Anamnese),
2. objektive Untersuchungsergebnisse (klinisch, Laboratoriums- und Testergebnisse) und
3. die endgültige Beurteilung durch den behandelnden Arzt berücksichtigt.

Eine ausführliche Interpretation der ermittelten Ergebnisse ist schwierig und bei der beschränkten Redezeit nicht möglich. Einige—wie uns scheint—wichtige zahlenmässige Ergebnisse werden daher hier auszugsweise in Tabellenform dargestellt, die vorerst nur als eine Diskussions- und Vergleichsgrundlage angesehen werden können.

Im Hinblick auf den *Erkrankungsbeginn* (= 1. Asthmaanfall), wie er bei der Erhebung der Anamnese angegeben wurde, ergibt sich bei der

Auskertung (vergleiche Abb. 1), dass das Asthma ganz allgemein ohne Rücksicht auf die Pathogenese bei Frauen etwa um 10 Jahre früher als bei Männern in Erscheinung tritt. Der maximale Gipfel liegt bei den Frauen zwischen dem 15. und 20. Lebensjahr, bei den Männern um das 30. Lebensjahr. Hier dürften hormonale Einflüsse von wichtiger Bedeutung sein.

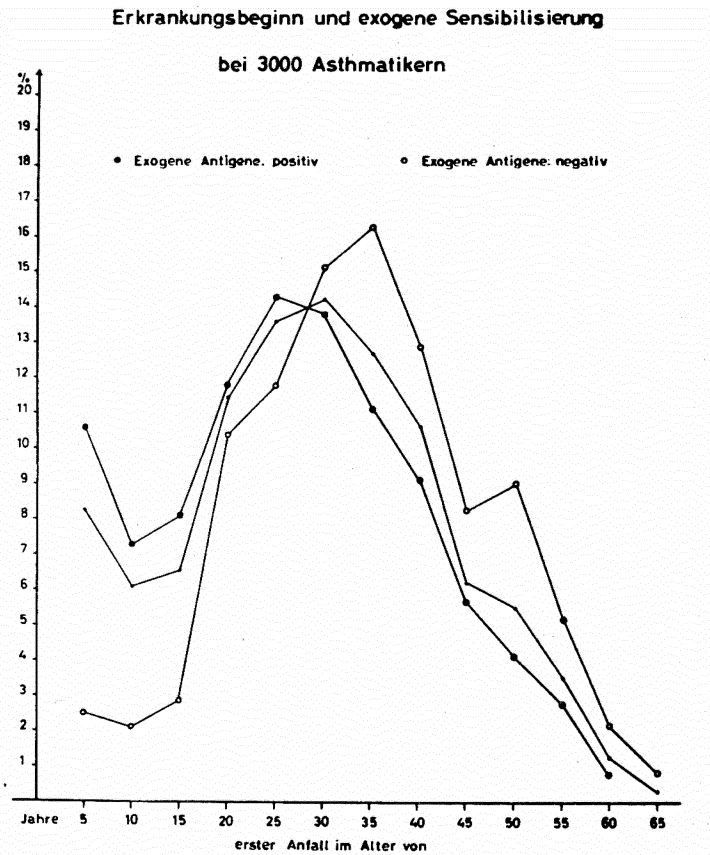

Abb. 2

In einem 2. Diagramm (vergleiche Abb. 2), wobei zunächst auf eine Unterteilung nach Geschlechtern verzichtet wird, zeigt sich sehr deutlich, dass Asthma bronchiale infolge Sensibilisierung gegen exogene Antigene früher und häufiger als Asthma *ohne* Sensibilisierung gegen exogene Antigene in Erscheinung tritt. Die beiden Gipfel liegen etwa um 10 Jahre auseinander. Für das Kindesalter ergibt sich ein weit häufigerer Asthmabeginn für die Sensibilisierten als für die Nichtsensibilisierten - eine Aussage, die uns recht wichtig erscheint und die Bedeutung der Sensibilisierung durch exogene Antigene im Kindesalter unterstreicht,

was zwar vermutet, aber bisher - zumindest in Deutschland - nicht ausreichend untersucht wurde. Bemerkenswert ist, dass für das Kindesalter das Geschlecht ohne Einfluss zu sein scheint. Für die Sensibilisierten zeigt sich bei Jungen und Mädchen etwa in gleicher Weise eine grössere Häufigkeit des Erkrankungsbeginns (vergleiche Abb. 3); für den Erkrankungsbeginn nach dem 15. Lebensjahr ergibt sich für Sensibilisierte wie Nichtsensibilisierte eine annähernd gleiche Häufigkeits-

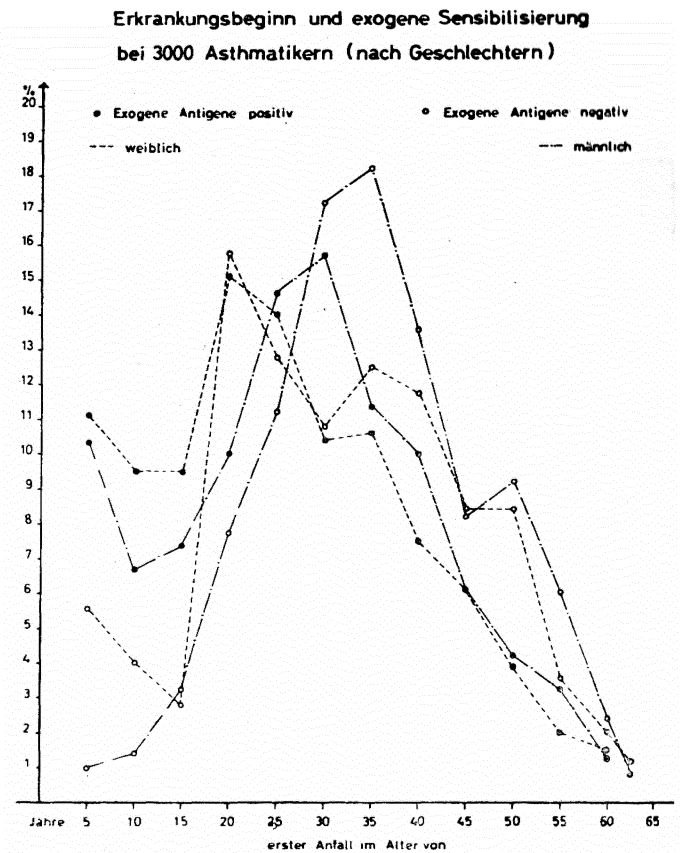

Abb. 3

werteilung, wobei die Frauen erkranken häufiger und früher als die Männer, besonders zwischen dem 15. und 20. Lebensjahr. Ferner zeigt sich bei den Männern eine Verschiebung des Gipfels bei den Sensibilisierten um 5 Jahre nach links, was auch in Abbildung 2 zum Ausdruck kam.

Primäre bzw. *sekundäre Realisationsfaktoren* wie bronchiales Reizsyndrom, Klimafaktoren, seelische Faktoren, Zyklusabhängigkeit bei Frauen und Sinussyndrom (vergleiche Abb. 4) zeigen eine fast gleiche

prozentuale Häufigkeit bei sensibilisierten und nichtsensibilisierten Asthmatikern.

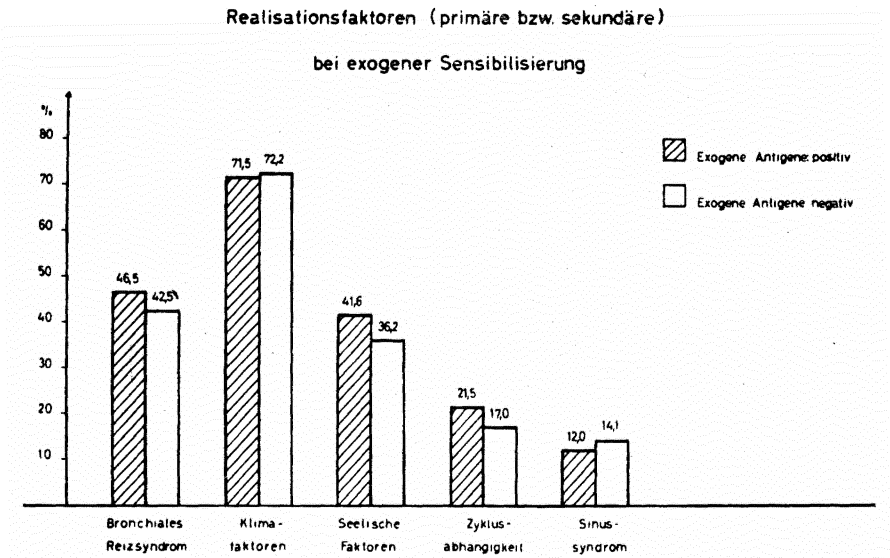

Abb. 4

Andere allergische Erscheinungen wie Urticaria, Ekzem, etc. treten bei sensibilisierten Asthmatikern doppelt so häufig als bei nichtsensibilisierten Asthmatikern auf, was der allgemeinen Erfahrung entspricht

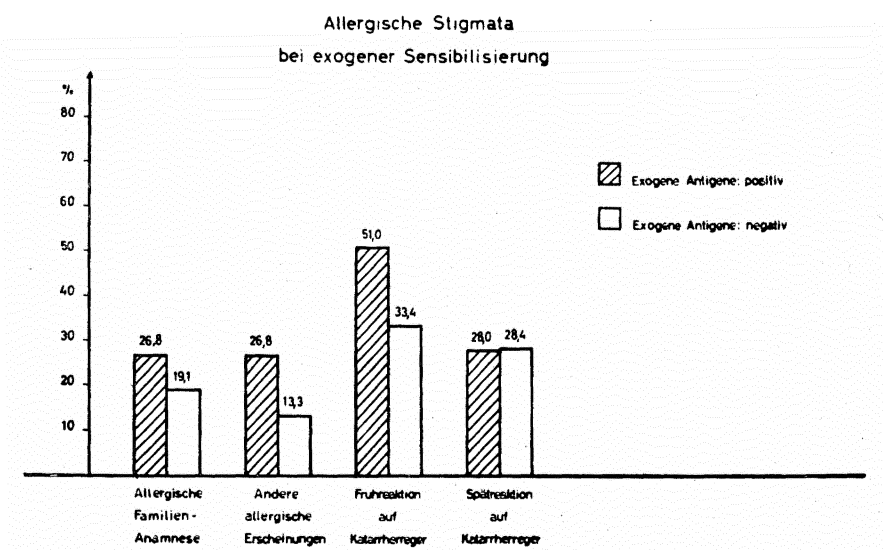

Abb. 5

(vergleiche Abb. 5). Interessant ist, dass eine positive *Frühreaktion auf Katarrherreger* wesentlich häufiger bei Asthmatikern mit einer Allergie gegenüber exogenen Antigenen festzustellen ist, während die Spätreaktion auf Katarrherreger bei Sensibilisierten wie Nichtsensibilisierten in gleicher Häufigkeit zu beobachten ist, m. a. W.: die Bereitschaft, nach dem anaphylaktischen oder Frühreaktionstyp zu reagieren, hat in einem Teil der Fälle auch für Entwicklung einer Infektallergie Bedeutung.

Ich fasse die Ergebnisse noch einmal kurz zusammen:

1. Das Asthma bronchiale tritt in der Bundesrepublik Deutschland bei Frauen etwa um 10 Jahre früher als bei Männern auf. Der Häufigkeitsgipfel liegt bei Frauen zwischen dem 15, und 20. Lebensjahr, bei Männern um das 30. Lebensjahr.
2. Exogen-allergisches Asthma bronchiale tritt früher und häufiger als Asthma ohne Sensibilisierung gegen exogene Antigene in Erscheinung.
3. Bronchiales Reizsyndrom, Klimafaktoren, seelische Faktoren, Zyklusabhängigkeit bei Frauen und Sinussyndrom als primäre bzw. sekundäre Realisationsfaktoren zeigen eine fast gleiche Häufigkeitsverteilung bei sensibilisierten und nichtsensibilisierten Asthmatikern.
4. Andere allergische Erscheinungen wie Urticaria, Ekzem, etc. sowie eine positive Frühreaktion gegen über Katarrherregern treten bei Asthmatikern mit Sensibilisierung gegen exogene Antigene wesentlich häufiger als bei nichtsensibilisierten Asthmatikern auf.

ZUR AETIOLOGIE DES ASTHMA BRONCHIALE IN DER SCHWEIZ

B. FUST

Auf Wunsch des Präsidenten unseres Kongresses, Prof. C. JIMÉNEZ DÍAZ, führte die Schweizerische Allergie-Gesellschaft eine Erhebung über die Ursachen des Asthma bronchiale in der Schweiz durch. Daran beteiligten sich die Kollegen

PD Dr. S. Cruchaud und Dr. J. Trolliet, Medizinische Universitätspoliklinik, Lausanne,

Dr. E. Musso, Dermatologische Universitätsklinik, Genf,

Prof. Dr. H. Storck und Dr. H. P. Huber, Dermatologische Universitätsklinik, Zürich, und

Dr. F. Wortmann, Allergie-Poliklinik der Dermatologischen Universitätsklinik, Basel,

denen ich für die geleistete Arbeit herzlich danke.

In *Basel* wurden im Jahre 1963, 502 Patienten wegen Asthma bronchiale, Bronchitis asthmatica oder "Asthma-Bronchitis" im Skarifikationsverfahren auf Sensibilisierung untersucht. Davon zeigten 324 oder 65 % insgesamt 468 Hautreaktionen, von denen 62 % auf Hausstaub (worunter 18% nur auf den eigenen Staub), 28% auf Pollen, 4% auf Schimmel und 6% auf andere Allergene (Mehl, Tierhaar, etc.) entfielen (Tabelle 1). Diese Verteilung stimmt mit den Ergebnissen ähnlicher Zusammenstellungen aus früheren Jahren gut überein und kann für das Einzugsgebiet von Basel als repräsentativ gelten. Die Frequenz der positiven Reaktionsausfälle nimmt mit zunehmendem Alter ab Sie sinkt von 80% bei Kindern und Jugendlichen, auf 21% bei den mehr als 60-Jährigen (Tabelle 2).

In *Genf* reagierten 57 von 65 Asthmatikern, d.h. 88 %, auf die intradermale Testung mit einem von Prüfer selbst zubereiteten Staubextrakt.

In *Lausanne* wurden in den Jahren 1953-1958 bei 1023 Asthmatikern intradermale oder cutane Proben angestellt mit 76 % positiven Ergebnissen, die weitaus am häufigsten durch Hausstaub bedingt waren. Von 182 Asthmapatienten mit positiven Hauttesten, über welche detailliertere Angaben vorliegen, sprachen 48,5 % nur auf Hausstaub, 16,5 % nur auf Pollen, 13 % auf Pollen + Staub, 11 % auf Staub + Bettzeug

TABELLE 1

Asthma, Schweiz

Basel, 1963: Von 502 Patienten zeigten 324 total 468 Hautreaktionen

	Davon % mit
Hausstaub	62
Pollen	28
Schimmel	4
Mehl, Tierhaar, etc.	6
Total	100

TABELLE 2

Asthma, Schweiz

Basel, 1963: Altersverteilung der Reaktionsausfälle

Alter	Patienten	Hautreaktion		
		Negativ	Positiv	%
0-15	186	37	149	80
16-25	109	22	87	80
26-40	102	50	52	51
41-60	77	47	30	39
> 60 J.	28	22	6	21
Total	502	178	324	
%	100	35	65	

(Federn, Rosshaar) und je 5,5 % auf Staub + Tierhaar bzw. Staub + Schimmel an (Tabelle 3).

In *Zürich* kamen in den letzten Jahren über 2000 Asthmakranke zur allergologischen Abklärung. Davon waren 400 aus den Jahren 1958-1960 Gegenstand einer eingehenden Untersuchung. Bei 256 oder 64 % dieser Patienten, und zwar am häufigsten bei Probanden im Alter von 11-30 Jahren, liessen sich durch intracutane Allergengaben 479 Sofortreaktionen auslösen, wovon 52 % durch häusliche Allergene (Staub, Federn, Tierhaare), 24 % durch bakterielle Allergene, 21 % durch Pollen, 2 % durch Mehle und knapp 1 % durch andere Nehrungsmittel (Tabelle 4). Bei häuslichen Allergenen stimmten Hauttest und Anamnese in 85 %, bei Pollen in 78 %, bei bakteriellen Antigenen jedoch nur in 18 % überein. Bei 25 Kranken mit Asthma bronchiale wurde der inhalative

TABELLE 3

Asthma, Schweiz

Lausanne: Von 182 Asthmatikern mit positiven Hauttesten reagierten

	%
88 nur auf Hausstaub	48,5
30 nur auf Pollen	16,5
24 auf Pollen + Staub	13
20 auf Staub + Bettzeug	11
10 auf Staub + Tierhaar	5,5
10 auf Staub + Schimmel	5,5
Total	100

TABELLE 4

Asthma, Schweiz

Zürich, 1958-60: Von 400 Asthmatikern zeigten 256 total 479 Hautreaktionen

	Davon % mit
Staub, Federn, Tierhaar	52
Bakteriellen Allergenen	24
Pollen	21
Mehlen	2
Anderen Nahrungsmitteln	1
Total	100

Antigen-Expositionstest nach *Gronemayer,* gemessen mit dem Pneumometer nach *Wyss-Hadorn,* mit 1-9 Allergenen durchgeführt (IAPT-Test). Er fiel bei 22 Patienten in 30 von 55 Expositionen positiv aus. 15-mal war er trotz positiven Hauttests negativ, jedoch nie positiv bei negativer Intracutanreaktion. Von 139 Asthmatikern reagierten 100 oder 72 % gut bis sehr gut auf die Desensibilisierung mit Inhalationsallergenen, wohingegen von 60 mit mikrobiellen Extrakten vaccinierten Patienten 33 oder 55 % gut bis sehr gut auf diese Behandlung ansprachen. Für die Genese des Asthma bronchiale sind, besonders bei jüngeren Patienten, Inhalationsallergene, vor allem häuslicher und pflanzlicher Provenienz, von aetiologischer Bedeutung.

THE ETIOLOGY OF BRONCHIAL ASTHMA IN THE UNITED STATES

L. N. GAY. (*)

Bronchial asthma occurs in all races, age and sex play no part in its selection of victims. Because asthma is an hereditary disease, location, soil, environment and climatic factors play a secondary role in the *onset* of symptoms. However, symptoms may be aggravated by any one of these factors. Occupation is so important that on occasions one must insist upon a complete severance of the patient from the work. One must mention the predisposing constitutional cause of asthma, heredity. It is the unanimous belief that the disease itself is not inherited, but that the underlying tendency of which asthma is an expression is transmitted to the patient.

As I analyze in retrospect the clinical material which I have had the opportunity to study, I am impressed with the importance of the classification which I have endeavored to follow in the final analysis of the cause of these symptoms. As soon as an etiologic factor has been determined, a positive decision can be arrived at. Then, treatment is either a success because of elimination of the cause or a failure because of the complexities of the disease.

It is essential for the clinician to realize that asthmatic symtoms are produced by extrinsic factors as well as by intrinsic factors. In other words, some material outside of the body of the patient but in his environment produces respiratory discomfort after its inhalation. Included in the extrinsic group is that large number of patients who, because of emotional strain, develop attacks of asthma.

The intrinsic factors are less tangible; the patient develops his symptoms after he has contracted an infection, such as sinusitis or chronic tonsillitis. In this group one must place the asthmatic who has developed emphysema associated with dyspnoea. Differential diagnosis is not difficult, provided the examination of the patient has been sufficiently thorough. The patients, who have been under my observation in private practice and in the clinic of the Johns Hopkins Hospital, are grouped under the following classification:

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ETIOLOGY-ASTHMA IN U. S. A.

1. Allergy-(eastern seaboard) following pollen inhalation, often the aftermath of years of pollen hay-fever: Trees-early spring.
Grasses-mid spring and early summer.
Ragweeds-August and September.
Added factors: Western area of the United States; other airborne pollens.
a. Complication following same: Upper respiratory infection, such as sinusitis. This leads to low grade bronchitis with associated dyspnoea and residual asthmatic bronchitis.
b. Unrecognized sinusitis, prolonged asthmatic bronchitis in individuals over 45 years, emphysema as the final condition.

2. Allergy.
a. Following the inhalation of organic dusts, whether animal danders, house dusts or industrial dusts, as the baker of bread (wheat) or the grain merchant who handles mixed feeds for animals. Thus they may react to an ingredient of the feeds, which may contain cottonseed, linseed, castor bean and many other high protein vegetable compounds.
b. The spores of fungi.

3. Non-Allergic Asthma.
a. This group develops asthma following long standing infection, the sinuses especially, ethmoids or antra, less frequently the sphenoids or tonsils.

4. Industrial Asthma.
a. Inhalation of irrigating fumes. Steer industry, chemical industry, where fumes of plastic fill the atmosphere. This type of asthma is not allergic, but is often associated with a specific chemical inhaled by the victim, who might also be an excessive smoker of cigarettes.
Hot-air heated homes, (fumes and dust from coal).
Pungent cosmetics, (perfumes).

5. Drugs. "Aspirin" and other coal tar products. Penicillin. Iodides, etc. Coal Tar: Sulfa or Iodides.

6. Less frequent etiologic factors:
a. Endocrine disorders.
b. Psychogenic disorders.
c. Cystic fibrosis.

7. Climatic factors.

8. Asthmatic Bronchitis in children due to residual or hypertrophied lymphoid tissue in the nasopharynx. Usually nocturnal symptoms, due to drainage of mucoid discharge into the trachea while child is sleeping.

ETIOLOGIC FACTORS OF BRONCHIAL ASTHMA IN ISRAEL

I. GLAZER.

Chief, Allergy Clinic, Tel-Hashomer Government Hospital, Ramat-Gan, Israel.

Israel (*) is a country whose population is the product of mass immigration of Jewish people from many continents. Its practicing allergologists, therefore, have a unique opportunity to observe allergic phenomena in different ethnic groups when these groups are exposed to a new environmental set-up with possibly new allergens. There are many instances of an entire isolated inbred community moving to Israel in the course of a few years, as did the Jews from Iraq, Yemen, Kutzin (India) and Bulgaria. Yet there is a marked difference of morbidity of bronchial asthma among these different communities. Suffice to mention the marked prevalence of this disease among immigrants from Iraq and Yemen.

In 1952-1953 the rate of hospitalization of asthmatic patients born in Iraq and Yemen was almost four fold and two fold larger respectively as compared to those born in Europe and America (Table I) [10]. However, in recent years we have noted a definite tendency for reduction in the severity of asthma and consequently the rate of hospitalization of Iraqis although their number in the Out-patient Clinic is still high [7].

(*) The state of Israel (fig. 1) comprises an area of 20,850 square kilometers (8,048 square miles) and is bounded by the Mediterranean Sea to the West and Lebanon and Syria to the North. The Sea of Galilee (Lake Tiberias) part of the Jordan river and the Dead Sea form part of its border with the Kingdom of Jordan to the East and South-East. Eilat on the Southern tip of the Negev is a small port city on the gulf of Akaba of the Red Sea. On the South-West border of the Negev is the Sinai peninsula of Egypt. Israel extends from 29° 30' to 33° 15' latitude North and from 30° 17' to 35° 41' East of Greenwich.

The main geographic and climatic regions of Israel are: (1,10).

1) The Mediterranean coast and coastal plain with an equable (sea) climate of warm humid summers and mild winters. Variations between day and night temperatures are moderate.

2) The Jordan Valley along the Jordan river from the Sea of Galilee (200 meters below sea level) to the Dead Sea (392 meters below sea level-lowest point on earth). Its climate is subtropical in nature.

3) The range of mountains and hills which is interposed between the above two zones. It has an inland type of climate: hot dry days and cool nights in the summer and relatively cold winters but no snow. The mountains of Galilee (Nazareth, 400 m.; Safad, 900 m.) are separated by the Plain of Esdraelon (Emeq-Yizrael) from the Mount Carmel, Shomron and Further South Judea (Jerusalem, the Capital, 800 m.)

4) The Negev comprises the Southern half of the area of the State with Beer-Sheva its main city. It is hilly barn desert-like with a dry hot climate and marked difference between day and night temperature.

TABLE I

Hospitalized asthma cases in 1952-1953 per 100.000 population, ages 45-49 yrs.

Place of birth	Rate of Hospitalized cases
Europe-America	34
Yemen	64
Iraq	125
Turkey	43
Morocco	40
Libya	17

The marked familial incidence of bronchial asthma among the Iraqui Jews in Israel may be explained in part by their tendency for inbreeding and cousin marriages for many centuries in their country of origin. Yet their atopy has become manifest mainly after they began living in Israel. When a careful history is taken one finds that even in Iraq some members of the family suffered from hay fever and/or asthma but these cases were mild and scarce indeed. Coming from a dry inland type of climate many of them settled in the humid Mediterranean coastal plain which favours the growth of molds and hay fever plants and the development of respiratory infections. It is quite interesting however to note the relatively low incidence of pollen skin reactions to pollen extracts in these allergic Rhinitis and asthma patients in their first years in Israel [10], [13]. There are of course social, economic and psychological contributing etiologic factors which aggravate and perpetuate bronchial asthma among the Iraquis as well as other new-comers from Asia, North Africa and the European Continent. Many patients who were in Concentration Camps in Europe lost their asthma at least temporarily. Some of those who survived the most brutal inhuman treatment and deprivation, both physical and mental, under the Nazi regime, came down with bronchial asthma, in many cases just after liberation from concentration camps [10] or after arrival in Israel. Exposure to new or more potent allergens could be blamed but on the other hand psychological stress and physical stress associated with adaptation to a new environment is too obvious to be ignored.

One of the climatic phenomena apparently affecting the asthmatic patient in Israel is the Khamsin (Sharav). The Khamsin [1] is an eastern or southern wind blowing from the deserts surrounding the country and producing a marked drop of the relative humidity which is more noted in the mountain region than in the coastal plains. The Khamsins are more prevalent in the spring and fall, may persist from a few hours to a week or more and are usually associated with a marked rise in temperature. The physiological effect is thought to be due to some electromagnetic changes in the atmosphere over and above the dryness and

heat of the air. Many asthmatics state emphatically that all of their symptoms disappear during a Khamsin. Others get their attacks particularly if sand storms develop. It has been observed that during Khamsin days there are high atmospheric pollen and mold spore counts [14]. However, the disappearance of the Khamsin may be so abrupt that even those

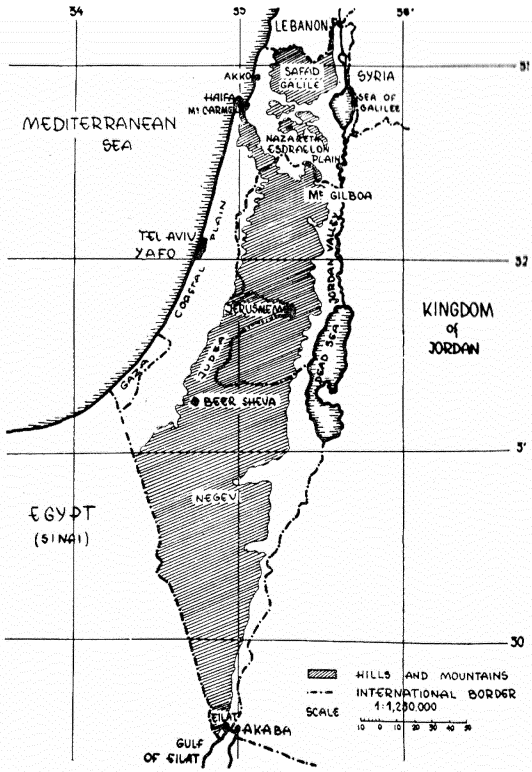

Fig. 1.—Map of Israel: Geographic and Climatic regions.

who feel well all through it come down with a seizure perhaps due to the sudden change in temperature and humidity. The Khamsin has no doubt an indirect late beneficial effect on pollen and mold asthma because the drying effect partially controls the growth of hay fever plants particularly grasses and reduces the development of molds.

Pollens constitute a relatively minor cause of asthma in Israel. In most cases of pollinosis, the asthma is subsequent to hay fever which is caused mainly by the Gramineae grasses (Table II).

Atmospheric pollen counts carried out by the gravity slide method in the coastal plain (Tel-Aviv-Jaffo [11, 12, 14] and Tel-Hashomer [5]) demonstrate the peak of pollination of the Gramineae to be in March or April (Fig. 2), which correlates well the severity of symptoms. This occurs usually about two to four weeks later in the colder mountain region (Jerusalem [4, 18], Nazareth) and somewhat earlier in the warm valleys

TABLE II

Grasses of the gramineae family causing hay fever and asthma in Israel

Botanical Name	Common English Name
Cynadon dactylon	Bermuda grass
Agrostis verticillata	Red top grass
Avena sterilis	Oat grass
Avena barbata	Oat grass
Dactylis glomerata	Orchard grass
Hordeum sativum	Cultivated barley
Hordeum murinum	Mouse barley
Lolium perenne	Perennial rye-grass
Lolium multiforme	Italium rye-grass
Poa annua	Annual meadow grass
Poa bulbosa	Meadow grass
Sorghum annuum	
Sorghum halepensis	Johnson grass

(Sea of Galilee and Jordan Valley [9, 14]). In the dry desert of the Negev and its Southern tip the City of Eilat [5], on the shore of the Red Sea, there is very little pollen indeed (fig. 2) and it is therefore a true haven for pollinosis patients.

During the dry summer season beginning late in May there is little pollination of hay fever plants in the entire country. Thus the tourist

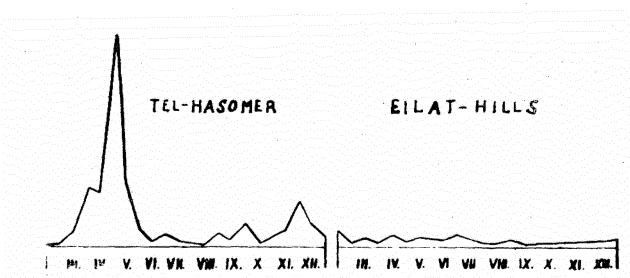

Fig. 2.—Typical gramineae grasses pollen weekly counts curves in the coastal plain (Tel-Hashomer) and the Negev Desert (Eilat Hills).

arriving in May from Europe and the Northern regions of the United States avoids the grass pollens both at home and in Israel.

In the fall with the very first rains there is a little rise in the Gramineae pollen count [5, 14] with minor symptoms [9]. In a few places Bermuda grass may pollinate and cause symptoms perennially [11, 14].

As to the composiate: Although there is some growth of *Ambrosia maritima,* there are none of the hay fever causing species. Americans sensitive to ragweed are therefore symptom-free in Israel [8]. Artemesia

(sage brush) which grows mainly in the coastal plain, was mentioned as a possible cause of hay fever [16]. Recently it was proven a cause of asthma in the fall [17].

Israel is devoid of natural forests, and hay fever trees are scarce indeed. Ailantus glandulosa (Tree of Heaven) was demonstrated to produce hay fever in Jerusalem [21], and in some rare cases trees like Acacia, Eucalyptus, Cupressus, Juniperus and Citrus were incriminated [13].

Olive tree pollen is a definite and quite important cause of hay fever in May [9, 11] and occasionally may even cause severe asthma [6].

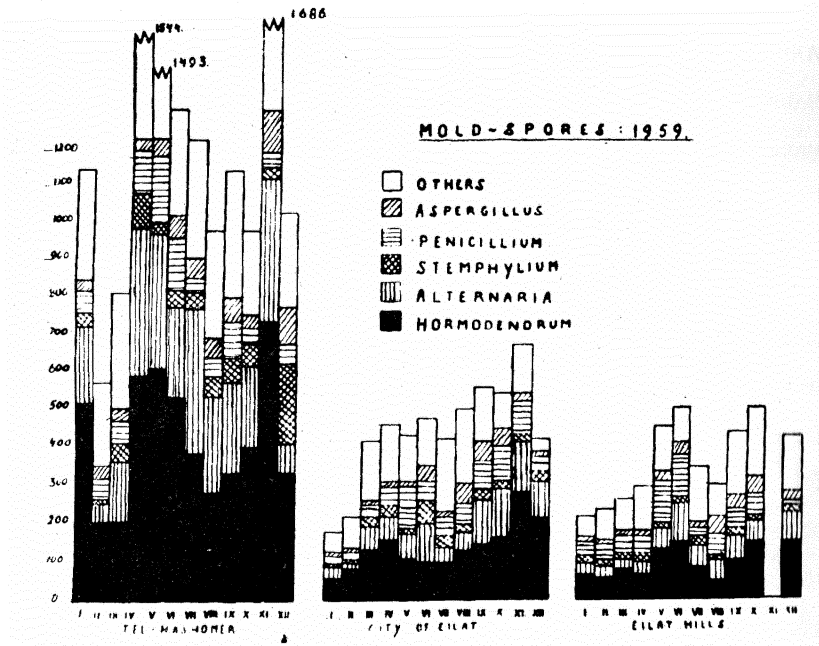

Fig. 3.—Total Monthly Number of colonies cultured from atmospheric mold spores in the coastal plain (Tel-Hashomer) and the Southern Negev (Eilat).

Mold spores are no doubt an important etiological factor for respiratory allergic diseases in Israel although there are no statistical clinical reports to that effect. Atmospheric spores identified and counted on slides in Tel-Aviv-Jaffo [11, 12, 14] show a distinct peak in the Spring (May) and a lesser one in the Fall. Culture plate studies in Tel-Aviv and other localities [2] have verified these seasonal variations. The principal source of the spores is the fungus Hormodendrum (Cladosporium). In a recent survey we were able to demonstrate [3] the paucity of atmospheric fungus spores in the Negev city Eilat when compared simultaneously with Tel-Hashomer in the coastal plain (fig. 3). Yet in both of these sites the genera cultured were similar and in the following order of frequency: Hormodendrum, Alternaria, Penicillium, Aspergillus, Stemphylium, Actinomycetes and Yeasts.

Other etiologic factors of bronchial asthma like house-hold inhalants, animal danders, insect proteins and stings or drugs are essentially the same as in other parts of the world and need no special discussion.

As in many other countries there are large groups of asthmatic patients in whom no etiologic factors can be definitely established by history or by routine skin tests and we assume that these cases are of bacterial origin. They comprise about half of our adult asthmatic patients.

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FREQUENCE DES FACTEURS ETIOLOGIQUES DANS L'ASTHME BRONCHIQUE EN BELGIQUE

J. M. JAMAR.

Louvain.

Il est souvent malaisé de déterminer avec précision chez un malade asthmatique l'importance relative des différents facteurs qui interviennent dans l'étiologie de sa maladie ou qui en influencent le cours.

Habituellement en effet on se trouve devant une origine complexe. Chez un pourcentage assez élevé il existe une base allergique mais son importance peut être fort variable. Alors que chez certains son influence est prédominante chez d'autres son rôle n'est plus que secondaire. En général au fur et à mesure que la maladie progresse ou que le sujet avance en âge l'importance du facteur allergique a tendance à diminuer tandis que l'irritation anspecifique des bronches augmente.

Chez la plupart des patients asthmatiques les infections des voies respiratoires aigues ou chroniques, bactériennes ou même virales, interviennent dans le déclenchement des symptômes de broncho-spasme et déterminent souvent le cours de la maladie. La fréquence, la durée et l'intensité des infections conditionnent généralement la déterioration progressive de l'état du malade.

Comme dans toute maladie chronique un changement s'opère fréquemment dans le psychisme du malade, ce qui influence défavorablement le cours de la maladie. A la longue le facteur psychique devient parfois le facteur le plus en vue dans le déclenchement des crises.

Il est toutefois difficile de faire une statistique sur la fréquence et l'importance de ce facteur puiqu'une influence psychique se manifeste à un crtain moment dans une mesure plus ou moins importante dans la majorité des cas.

FACTEURS ALLERGIQUES.

Dans une étude portant sur 2.000 cas d'asthme non sélectionnés, il est apparu qu'on peut mettre en évidence une étiologie allergique partielle ou totale, dans 68 % des cas, tandis que dans 32 % aucune allergie n'était décelable (fig. 1).

Dans la majorité des cas toutefois le facteur allergique ne pouvait être considéré comme étant le seul en cause et son importance déterminante sur le cours de la maladie peut être variable suivant les cas.

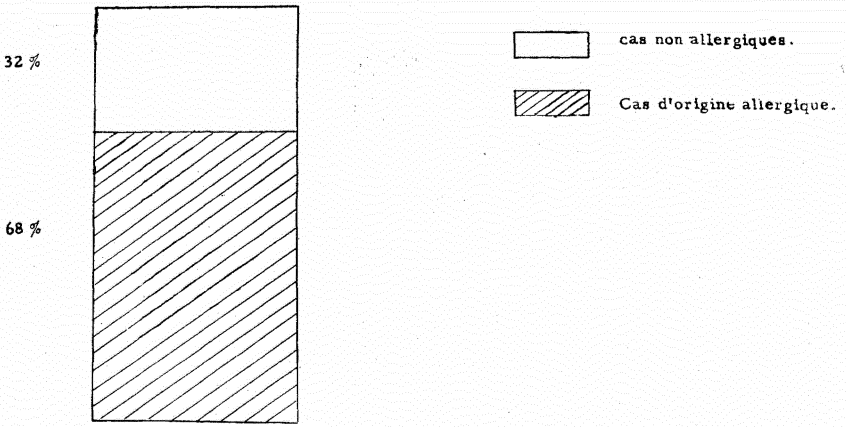

Fig. 1.—Malades asthmatiques allergiques et non allergiques.

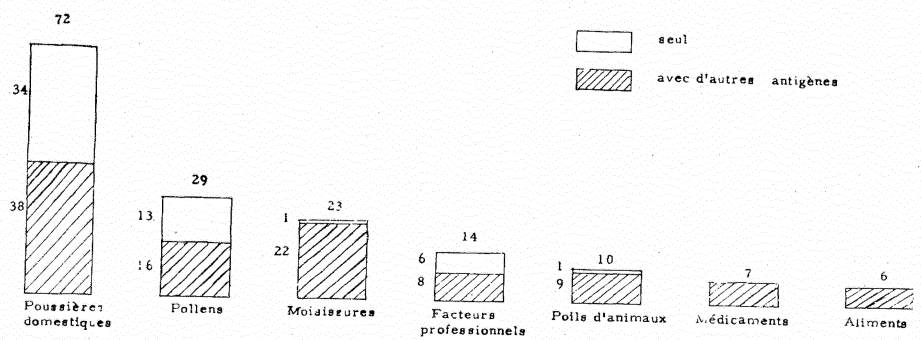

Fig. 2.—Facteurs étiologiques dans 1.000 cas d'asthme d'origine allergique.

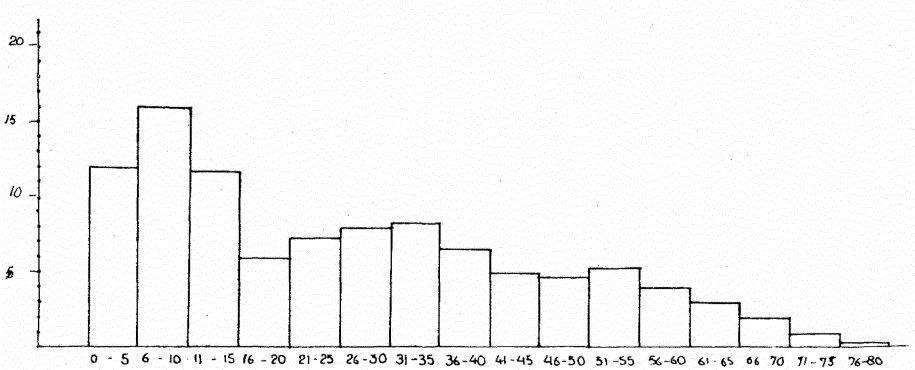

Fig. 3.—L'âge des patients.

Les sujets présentant des manifestations allergiques se rencontrent surtout entre l'âge de 6 à 40 ans tandis que les non-allergiques sont trouvés essentiellement parmi les tout jeunes ou chez ceux ou la maladie a débuté après l'âge de 40 ans.

Les critères employées pour mettre en évidence une allergie sont :

— le rèsultat des tests cutanés éventuellement complétés par les tests d'inhalation.

— la relation de ceux-ci avec les données de l'interrogatoire.

— le résultat de l'élimination et ou de la désensibilisation.

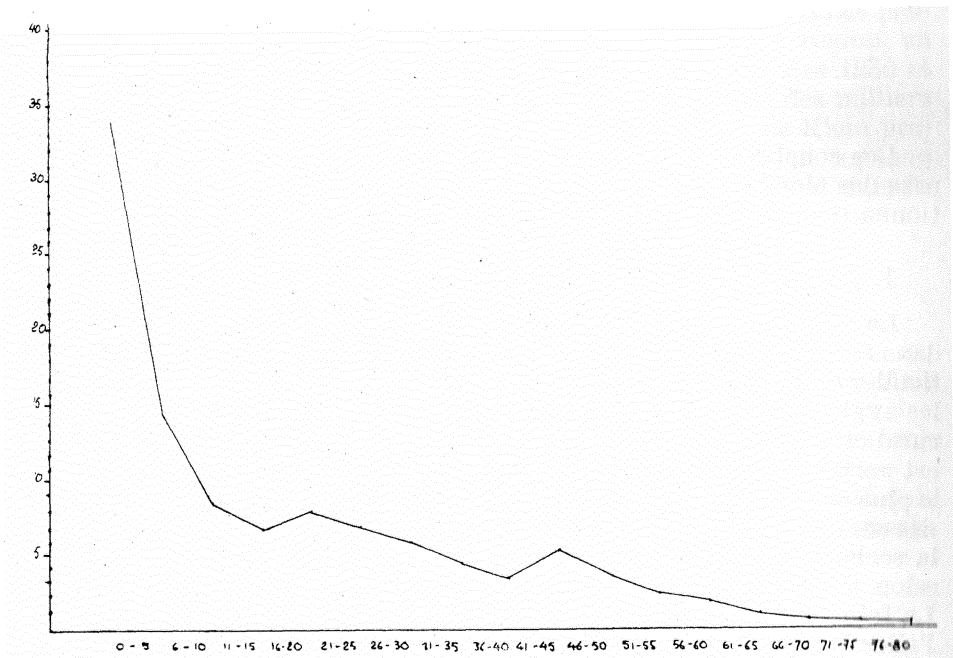

Fig. 4.—L'âge du debut de la maladie.

Dans la catégorie des malades allergiques la liste des différents antigènes trouvés responsables est montrée dans la fig. 2. (Etude portant sur 1.000 cas non sélectionnés.)

L'âge des patients étudiés, ainsi que l'âge du début de la maladie, sont montrés dans les figures 3 et 4. Ces données peuvent en effet avoir un intérêt si on veut comparer différentes statistiques. Il est en effet certain que l'image peut changer considérablement suivant que l'étude porte sur des enfants des adultes ou des vieillards. Nos cas se composent surtout d'enfants et d'adolescents dont le début de la maladie est assez récent.

FREQUENCE DES FACTEURS ETIOLOGIQUES DE L'ASTHME BRONCHIQUE EN FRANCE

R. KOURILSKY.

Les conclusions de ce travail sont fondées sur une enquête faite auprès des Membres de la Société Française d'Allergie, au moyen de questionnaires détaillés.

1. *Etude des allergènes principaux.*

La poussière de maison est le principal. Cette prédominance est notée dans toutes les régions, elle est maxima dans les centres urbains, en particulier dans les très grandes villes (63 à 78 %), légèrement moindre dans les agglomérations moyennes (60 à 52 %), nettement moindre en milieu rural (40 %). L'allergie aux plumes vient en deuxième lieu, sa fréquence est variable selon les régions (de 20 à 30 %). Elle est rarement isolée, et le plus souvent associée avec l'allergie à la poussière (dans les trois quart des cas). L'allergie aux moisissures est plus souvent décelée depuis qu'on la recherche systématiquement, mais sa fréquence relative est variable selon les régions (5 % dans les villes, jusqu'à 30 % en milieu rural). La fréquence de l'allergie à Candida Albicans est diversement appreciée; l'allergie aux textiles (laine, kapok) est relativement rare.

L'allergie bactérienne est difficile à chiffrer exactement. Elle est en général considérée comme étant peu fréquente (5 %), consécutive à l'infection secondaire d'un asthme primitivement non infecté et constatée surtout chez l'enfant. Cependant son rôle pathogene est important.

Il est de plus en plus clair que l'infection est un des plus puissants modificateurs de la paroi bronchiolaire. Jointe à l'allergie, elle a pour conséquence d'orienter l'évolution des asthmes infectés vers l'emphysème irréversible. Sa signification pronostique est donc sérieuse (R. KOURILSKY, HALPERN).

Il apparaît de même que l'allergie au tabac et aux produits de combustion des cigarettes joue un rôle de plus en plus reconnu dans l'évolution vers l'emphysème (R. KOURILSKY), chez les sujets génétiquement prédisposés.

Sa conjugaison avec le facteur précédent est particulièrement redoutable.

Les pollens sont rarement responsables de l'asthme bronchique proprement dit, mais ils constituent l'allergène majeur de la saison estivale:

les graminées sont responsables dans toutes les régions (76,8 %) ; à un bien moindre degré les composées (20 à 36 %), les arbres (10 %), avec d'assez grandes variations régionales.

Les poils d'animaux sont plus fréquemment en cause en milieu rural, à un bien moindre degré en milieu urbain (poils de chat 6,5 %, ou de chien 8 %), on les trouve chez 5/100 des malades présentant des tests positifs. Les autres allergènes de cet ordre sont exceptionnellement rencontrés. La poussière de meunerie l'est surtout en milieu rural (14,5 %), de même que les poussières de céréales (blé, orge, avoine).

Il n'y a pas de doute que de subites concentrations en allergènes peuvent faire éclore pendant un certain temps, dans une certaine région, un nombre appréciable de cas supplémentaires. C'est ansi qu'entre 1950 et 1961 une véritable "épidémie" sévit à Marseille, elle toucha des milliers de personnes. Elle était due à une poussière de tourteaux de Ricin provenant du port et surtout des usines environnantes. Il fallut un long procès aux plus dangereuses de ces usines pour tarir la source. Mais de tels cas sont rares.

2. *Les influences régionales.*

L'asthme est une maladie fréquente dans la plupart des régions urbaines surtout, et dans certains districts ruraux de climat humide. Mais sa fréquence générale ne paraît pas augmenter.

L'évolution vers l'infection surajoutée est souvent constatée en milieu urbain (33 à 60 %), surtout en hiver, 15 % seulement en été.

La proportion des asthmes continus est relativement faible (2 à 15 %), celle des asthmes grave est diversement appréciée (20 à 40 %). Les asthmes mortels sont heureuxement rares (1/700 à 1/1000).

3. *Influence de l'âge.*

La proportion de l'asthme infantile est forte (30 à 40 %) suivant les conditions d'observation; 10 % chez l'adolescent (12 à 20 ans) ; 32 % chez l'adulte; 12 % au-delà de 50 ans.

4. Parmi les *facteurs étiologiques,* de récents travaux ont montré que le facteur psychologique individuel, et en particulier l'existence de conflits affectifs, est un facteur décisif dans la modulation évolutive de l'asthme, son déclanchement, son intensité et sa durée. Ce facteur est accessible à la thérapeutique, ce qui permet une réduction considérable de la corticothéraphie en durée et en quantité.

Ce facteur étiologique n'a pas été considéré à sa juste valeur, il est pourtant très répandu. Conjugué aux allergènes ambiants, il explique la pérennité de l'asthme. Il n'est pas encore possible de savoir s'il correspond à un facteur génétique différent de celui qui gouverne l'hypersensibilité allergique.

FACTORES ETIOLOGICOS DEL ASMA BRONQUIAL EN ESPAÑA

F. LAHOZ, A. SASTRE, J. PÉREZ GUERRERO, F. MARÍN y R. DOMÍNGUEZ

Fundación Jiménez Díaz (Clínica de Nuestra Señora de la Concepción). Departamento de Asma y Enfermedades Alérgicas.

Para establecer lo más exactamente posible los factores etiológicos del asma en un país, es necesario recurrir al análisis de casuísticas amplias de diversas áreas dentro del país en cuestión. Ahora bien, las estadísticas examinadas deben reunir algunas condiciones que permitan sacar deducciones seguras. Nos referimos a la conveniencia de una homogeneidad en los métodos que han llevado al diagnóstico del enfermo con el que luego va a figurar en la estadística. Es evidente que, si entre dos observadores, uno utiliza sólo la clínica, y otro, además de ésta, la exploración funcional, es más probable que el segundo realice una separación más exacta de los asmáticos, en relación con otros enfermos afectos de enfisema, fibrosis pulmonares, etc., cuya disnea semeja, en ocasiones, al asma bronquial. Lo mismo podemos decir en lo que se refiere a la metódica para demostrar la sensibilización. Lógicamente los resultados serán distintos según se valore las pruebas cutáneas, se utilicen extractos de diferentes procedencias, más o menos segura, o se practiquen pruebas de provocación casi sistemáticamente. Indudablemente, no debemos comparar estadísticas en las que el proceder seguido en el estudio de los enfermos no haya sido similar. Por eso nosotros vamos a basar este trabajo en nuestra propia casuística del Departamento de Alergología de la Fundación "Jiménez Díaz", donde hemos visto desde 1955 a 1963, 3.303 enfermos asmáticos, en colaboración con los doctores SASTRE, PÉREZ GUERRERO, MARÍN y DOMÍNGUEZ, ya que de esta forma manejaremos un grupo de enfermos en los que el criterio de selección y diagnóstico es el mismo para todos ellos. Basados en estas razones de homogeneidad no incluímos aquí enfermos vistos por nuestro grupo en épocas anteriores, que han sido analizados en otras ocasiones desde el punto de vista estadístico [1-2], y que si bien harían la casuística más numerosa, introduciría cambios en la metódica de estudio, especialmente en lo que se refiere a la pesquisa de la sensibilización y exploración funcional respiratoria. Por otro lado, al trabajar nosotros en Madrid por su situación central, nuestros enfermos proceden de diversos puntos de España, y por ello las conclusiones que saquemos no serán de un carácter puramente localista. Na-

turalmente que para completar esta visión panorámica del asma en España haremos mención de observaciones de otros colegas que, amablemente, nos han sido facilitadas.

Material y métodos

Hemos seleccionado un conjunto que reúne las siguientes condiciones: a) Presentar franca reacción asmática, tanto desde el punto de vista clínico como funcional respiratorio. b) Corresponder a un mismo nivel social, por lo que excluímos los casos pertenecientes a la consulta privada. c) Investigación cuidadosa de la sensibilización por metódica similar en todos ellos. En este sentido hemos de destacar que no sólo hemos utilizado la exploración cutánea, sino también las pruebas de provocación, según hemos presentado en otro lugar [3]. En lo que se refiere a los alergenos probados, hemos ampliado algunos de ellos, como los insectos, y mejorado la técnica de obtención de otros, como el polvo de casa, de acuerdo con lo expuesto en otros trabajo de nuestro grupo [4]. En el examen detallado de cada caso hemos adoptado un criterio especialmente severo para admitir la sensibilización. En todos ellos se han especificado las razones de alergenicidad, comprendiendo bajo este epígrafe la anamnesis, pruebas cutáneas, congruencia o no entre ambas, transferencia pasiva y pruebas de provocación en el caso en que hubieran sido realizadas. No hemos admitido nunca una posible sensibilización a no ser que reuniera por lo menos dos de las razones indicadas.

Con las condiciones reseñadas hemos reunido 3.303 enfermos en los que estudiamos no sólo la etiología, sino también el sexo, herencia y eosinofilia en relación con la misma.

El diagnóstico diferencial entre asma bronquial primario y secundario lo realizamos por medios clínicos y de exploración funcional, según fue ya comunicado en otro lugar [5]. La herencia ha sido analizada en padres, abuelos y tíos para las siguientes enfermedades: "asma, catarro habitual descendente, bronquitis, dermopatía (urticaria, eczema) y jaqueca".

Resultados y conclusiones.

Para mayor brevedad los exponemos en forma de cuadros. Si observamos los cuadros I y II, de su análisis puede deducirse, como del total de enfermos con disnea del tipo de reacción asmática, que asciende a 3.303 pacientes, el 36,5 por 100 después del adecuado estudio, fue diagnosticado de reacción asmática secundaria a enfermedad orgánica broncopulmonar. Los enfermos con auténtico asma bronquial o reacción asmática de base funcional y alérgica forman un total de 2.099. De éstos, el 43,1 por 100 son casos en los que no se ha podido objetivar sensibilización alguna, y que reúnen los caracteres clínicos del asma bacteriano. La cuarta parte de los asmas primarios son debidos exclusivamente a un exoalergeno que realiza la enfermedad por un mecanismo antígeno-

CUADRO I

NÚMERO TOTAL DE ENFERMOS	3.303
Asma bronquial	2.099 = 63,5 %
Asma bronquial secundario (bronquitis-fibrosis-enfisema-bronconeumopatía crónica)	1.204 = 36,5 %

CUADRO II

Asma bronquial

Bacteriano	903 = 43,1 %
Alergénico (sensibilización demostrada como única etiología)	524 = 25,0 %
Mixto (Bacteriano y Alergénico)	672 = 31,9 %

anticuerpo, quedando, por tanto, epigrafiados como asmas alergénicos. Por último, queda un 31,9 por 100 de los enfermos con asma bronquial primario de etiología mixta, bacterianos y alergénicos, ya que en ellos se ha podido demostrar una sensibilización que, sin ser decisiva, influye evidentemente desde el punto de vista causal y sintomático del paciente.

Hemos analizado en nuestra casuística la distribución del sexo en cada uno de los cuatro grandes grupos etiológicos (bacteriano-alergénico-mixto y secundario), como puede verse en el cuadro III.

CUADRO III

Sexo		Asma bacteriano	Asma alergénico	Asma mixto	Asma secundario
Varón...	1.738 = 52,6 %	450 = 49 %	222 = 42,4 %	286 = 42,6 %	780 = 64,7 %
Hembra.	1.565 = 47,4 %	453 = 51 %	302 = 57,6 %	386 = 57,4 %	424 = 35,3 %

Según se deriva de la observación de este cuadro, no existe preferencia en cuanto al sexo si consideramos los enfermos en su conjunto. Ahora bien, si los separamos en los grupos etiológicos indicados, no cabe duda de la existencia de una evidente preferencia por el sexo masculino en la reacción asmática secundaria, y un cierto predominio del femenino entre los asmas alergénicos, siendo ambos hallazgos significativos estadísticamente considerados.

Es de gran interés desglosar la etiología de los asmas alergénicos para ver la frecuencia de las diferentes sensibilizaciones demostradas, según hacemos en el cuadro IV.

Separamos en este cuadro los casos en los que sólo comprobamos una sensibilización de aquéllos con sensibilizaciones múltiples. Dentro de las monosensibilizaciones, es el polvo de casa el que destaca, pues llega a ser el 45,2 por 100 de todos los asmas alergénicos puros. Le siguen después los polínicos, que suman el 19,1 por 100. A continuación vienen el asma por hongos de ambiente y parásitos de los cereales, con el 4,8 por 100 y 3,8 por 100, respectivamente. Por último, y ya en pro-

CUADRO IV

ASMAS ALERGÉNICOS PUROS	524 = 25,0 %
a) *Mono-sensibilizaciones*	428 = 81,7 %
1. Asma doméstico (polvo de casa y clinoalergenos)	237 = 45,2 %
2. Asma polínico (gramíneas y árboles)	100 = 19,1 %
3. Asma por sensibilización a hongos de ambiente	25 = 4,8 %
4. Asma por sensibilzación a hongos parásitos de cereales	20 = 3,8 %
5. Asma por sensibilización a polvo de cereales	18 = 3,4 %
6. Asma climático (costas)	7 = 1,3 %
7. Asma por sensibilización a insectos	6 = 1,1 %
8. Asma por sensibilización a drogas	5 = 0,95 %
9. Asma por sensibilización a alimentos	4 = 0,76 %
10. Asma por sensibilización a productos dérmicos animales	3 = 0,57 %
11. Asma por parasitosis intestinal (ascaris)	2 = 0,38 %
12. Asma psicógeno	1 = 0,19 %
b) *Poli-sensibilizaciones*	96 = 18,3 %
1. Asma por sensibilización a polvo de casa y hongos de ambiente	38 = 7,2 %
2. Asma por sensibilización a polvo de casa y polen	19 = 3,6 %
3. Asma por sensibilización a polvo de casa y polvo de cereales	14 = 2,7 %
4. Asma por sensibilización a polvo de casa y alimentos.	12 = 2,3 %
5. Asma por sensibilización a polen y hongos de ambiente	3 = 0,57 %
6. Asma por sensibilización a polvo de casa y hongos parásitos de cereales	2 = 0,38 %
7. Asma por sensibilización a polen y drogas	2 = 0,38 %
8. Asma por sensibilización a polen y polvo de cereales	2 = 0,38 %
9. Asma por sensibilización a polen y alimentos	2 = 0,38 %
10. Asma por sensibilización a hongos de ambiente y polvo de cereales	1 = 0,19 %
11. Asma por sensibilización a hongos de ambiente y alimentos	1 = 0,19 %

porción menor, pueden verse otras etiologías, que aunque interesantes de demostrar desde el punto de vista terapéutico, son mucho menos frecuentes.

Los casos con asma alergénico puro, en los que se pudo objetivar más de una sensibilización, ascienden al 18 por 100 de los mismos. La polisensibilización más frecuente fue la del polvo de casa con otro alergeno como los hongos de ambiente (7,2 por 100) o el polen (3,6 por 100).

En el cuadro V presentamos los enfermos de asma mixto, bacteriano y alergénico que, como ya hemos indicado, son el 31,9 por 100 del total de nuestra estadística. En este grupo, igual que pasaba en los alergenos puros, es la sensibilización al polvo de casa la más frecuentemente asociada (67,5 por 100), siguen después los enfermos con sensibilización micógena (12,1 por 100) y polínica (7 por 100), pero como puede verse, en proporción bastante menor a la doméstica. Otros alergenos que, aunque menos frecuentes, intervienen etiológicamente en estos enfermos de asma mixto, son: el polvo de cereales (4,2 por 100), drogas (1,8 por 100), alimentos (1,2 por 100) y hongos parásitos de cereales (1,05 por 100).

Según podemos ver en el cuadro VI, la herencia, expresión de constitución disreactiva, es evidente. El 54,5 por 100 de los asmas prima-

CUADRO V

ASMAS MIXTOS		672 = 31,9 %
1.	Asma bacteriano con sensibilización alergénica doméstica.	453 = 67,5 %
2.	Asma bacteriano con sensibilización a hongos de ambiente.	81 = 12,1 %
3.	Asma bacteriano con sensibilización a pólenes	47 = 7,0 %
4.	Asma bacteriano con sensibilización a polvo de cereales	28 = 4,2 %
5.	Asma bacteriano y clima (costas)	14 = 2,1 %
6.	Asma bacteriano con sensibilización a drogas	12 = 1,8 %
7.	Asma bacteriano con sensibilización a polen y polvo de cereales	8 = 1,2 %
8.	Asma bacteriano con sensibilización a alimentos	8 = 1,2 %
9.	Asma bacteriano con sensibilización a hongos parásitos de cereales	7 = 1,05 %
10.	Asma bacteriano con sensibilización a hongos de ambiente y polvo de casa	6 = 0,9 %
11.	Asma bacteriano con sensibilización a polvo de casa e influencia psíquica	4 = 0,6 %
12.	Asma bacteriano con sensibilización a polvo de casa y polvo de cereales	2 = 0,3 %
13.	Asma bacteriano con sensibilización a polvo de casa y polen.	2 = 0,3 %

CUADRO VI

	Asma bacteriano	Asma alergénico	Asma mixto	Asma secundario
Herencia	60,5 %	49,0 %	56,0 %	32,0 %

rios presenta antecedentes alérgicos entre sus familiares. Resalta cómo los enfermos de asma bacteriano tienen mayor porcentaje de herencia que los alergénicos.

Otro atributo de la constitución alérgica es la eosinofilia, que de acuerdo con el cuadro VII, se demuestra en el 43 por 100 de nuestros casos de asma bronquial primario. Tanto la herencia como la eosinofilia tienen una incidencia menor en el asma secundario, por lo que pueden ser útiles para el diagnóstico diferencial.

CUADRO VII

	Asma bacteriano	Asma alergénico	Asma secundario
Eosinofilia (más de 4 %)	39,1 %	47 %	22,2 %

Debemos completar este estudio de la etiología del asma en España comentando brevemente algunas estadísticas que nos han sido facilitadas por otros alergólogos de nuestra patria, por lo que les manifestamos desde aquí nuestro agradecimiento por su colaboración y ayuda.

De Madrid hemos recibido estadísticas de los doctores C. LAHOZ, LÓPEZ BARRANTES y SALGADO, que no especificamos por ser en todo coincidente con la que acabamos de presentar. También de nuestra capital nos han enviado una amplia casuística los doctores CALVO FERNÁNDEZ y LÓPEZ PALACIOS, de 5.577 asmáticos, en la que destaca el predominio de los casos de asma bacteriano frente a los alergénicos, que son sólo el 9,9 por 100 del total. El doctor BOTELLA, de Madrid, ha examinado estadísticamente 4.000 asmáticos de su experiencia, resaltando la coincidencia de sus cifras con las nuestras, tanto en lo que se refiere al número de reacciones asmáticas secundarias a procesos orgánicos broncopulmonares, que llega al 39 por 100 de sus casos, como a la incidencia del asma debido exclusivamente a una sensibilización demostrada o asmas alergénicos, que son sólo el 27 por 100 de sus enfermos. El doctor TORRES ACERO, también de Madrid, nos ha enviado los factores etiológicos de 3.342 de sus enfermos, y así como coincide con nosotros en el bajo procento de asmas alergénicos (22,2 por 100), difiere en el franco predominio de asma primario, frente al secundario, dentro de la reacción asmática, pues los primeros llegan a 93,7 por 100 del total. Asimismo existe otra diferencia con nuestra estadística en la incidencia de la sensibilización micógena, ya que la objetiva en el 20 por 100 de los asmas alergénicos. Desde Zamora nos envía datos de su casuística personal el doctor VALDÉS, destacando en éstos: a) Frecuencia mucho mayor de las reacciones asmáticas secundarias en relación con el asma bronquial primario, ya que éste arroja un porcentaje del 8,5 por 100 del total de enfermos examinados por él, con disnea asmática. b) Porcentaje muy elevado de los asmas alergénicos. c) Frecuente hallazgo de polisensibilizaciones, siendo los cereales y sus parásitos el alergeno más

frecuentemente demostrable (34,9 por 100). El doctor A. OEHLING, de Pamplona, nos ha enviado parte de la estadística de su Servicio de Alergología de la Universidad de Navarra, que no reproducimos por ser muy similar a la nuestra, tanto en su procento de asmas debidos exclusivamente a un alergeno no bacteriano (12 por 100), como a los sensibilizantes que encuentra, siendo el más frecuente el polvo de casa. De las costas de España nos envían datos el doctor MORAGUES, de Málaga; el doctor LA CHICA, de Melilla; el doctor PONCE ARENCIBIA, de Las Palmas, y el doctor LÓPEZ RUIZ, de Mallorca. Los resultados obtenidos en Málaga y Melilla son muy similares entre sí y coincidentes con los presentados por nosotros, resaltando sólo una mayor incidencia de asma por hongos en relación con nuestra casuística. En Canarias, el doctor PONCE estudia 1.008 asmáticos, sobresaliendo entre ellos como peculiaridades regionales la existencia de casos por sensibilización al polvo de plátano, polvo de tabaco y pólen de pino de Canarias. El doctor LÓPEZ RUIZ subraya los siguientes puntos más sobresalientes del asma de Mallorca, después de revisar 3.850 enfermos: a) Gran frecuencia del asma con alto porcentaje de herencia en esta enfermedad, lo cual puede atribuirse al cruzamiento de sucesivas generaciones de familiares próximos. b) La gravedad de las crisis asmáticas (11 casos de muerte entre el total de enfermos). c) Incidencia grande de asmas alergénicos (54 por 100). d) Aproximadamente la mitad de los casos de polinosis son por sensibilización a parietaria oficinalis.

Como conclusiones etiológicas del asma en nuestro país, podemos decir:

1. Sólo un 25 por 100 de los casos son debidos a un alergeno como única causa responsable. O lo que es igual, solamente en la cuarta parte de los casos puede demostrarse un mecanismo inmunológico de importancia causal de la enfermedad.

2. En el 31 por 100 de los asmáticos restantes, puede encontrarse una sensibilización, pero ésta no es decisiva desde el punto de vista etiológico.

3. El alergeno más importante, por su frecuencia, es el polvo de casa, que solo o asociado es responsable de 60 por 100 de los casos de asma alergénico. Aproximadamente en la cuarta parte de ellos objetivamos una sensibilización al pólen, siendo más frecuente la de gramíneas, y menos los árboles, destacando entre éstas las del olivo.

4. Sigue después la sensibilización micógena aunque ya con una incidencia mucho menor. Encontramos un 5 por 100 de enfermos con sensibilización para hongos de ambiente, especialmente a Cladosporium, Penicillium y Alternaria, y en 7 por 100 de los casos se asocia ésta al polvo de casa. Los hongos parásitos de cereales (Tilletia y Ustílago) y los propios cereales ocupan el 7 por 100 de los casos. Otros sensibilizantes como los insectos, drogas, alimentos, caspas y productos dérmicos de los animales, los encontramos en proporción mucho menor.

5. Es evidente la importancia del factor constitucional que se demuestra en el 55 por 100 de los asmáticos examinados.

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FRÉQUENCE DES FACTEURS ÉTIOLOGIQUES DE L'ASTHME BRONCHIQUE EN CANADA

J. LÉGER.

Le Canada est un immense pays qui est bien connu universellement pour les rigueurs de son hiver; nombreux sont ceux d'autre part qui ignorent totalement que les canadiens sont également soumis à une saison estivale dont la température est parfois presque aussi rigoureuse que dans les contrées tropicales.

Ce dernier facteur ajouté au fait qu'il se trouve en Amérique une incidence extrêmement forte de pollens de ragweed, fait en sorte que le climat canadien est susceptible de favoriser l'éclosion et l'évolution d'un nombre très considérable de syndrômes asthmatiques allergiques, lesquels revêtent chez-nous une importance toute particulière. En effet, si un peu partout dans le monde il est un fait reconnu que la pollinisation est un facteur appréciable dans la sensibilisation des muqueuses des voies respiratoires, il faut reconnaître que le pollen de ragweed a des propriétés allergéniques toute particulières qui en font un agent sensibilisant de toute première importance. Nous avons donc au Canada des syndrômes de rhinite et d'asthme polliniques qu'il ne nous est pas permis de ne pas prendre au sérieux parce que dépendant d'un agent étiologique particulièrement actif.

A ce premier élément de l'incidence et de l'intensité de la pollinose du ragweed, il faut ajouter le fait que cette saison de pollinisation est brusquement suivie de changements drastiques dans la température canadienne et que à tout le moins dans une très vaste proportion du territoire, nous passons presque du jour au lendemain d'une température estivale à une température hivernale, de telle sorte que, il se trouve chez-nous des dizaines de milliers de malades allergiques souffrant de troubles respiratoires des voies supérieures et même de malaises bronchiques et pulmonaires, parce que soumis à l'exposition d'agents allergéniques puissants qui fait en sorte que leurs muqueuses sont congestionnées, irritées, et qu'elles sont pour autant très susceptibles à des variations de température importantes, donnant lieu très souvent à des infections qui sont d'autant plus mal supportées par les allergiques, que leur tractus respiratoire était déjà en état de déficience.

La conséquence de cette combinaison de facteurs, est que nous devons assister fort souvent à l'évolution d'un syndrôme allergique pur, qui normalement devrait connaître une rémission spontanée, mais qui

chez-nous donne lieu à des complications très sevères et très fréquentes telles la sinusite, la trachéobronchite, la pneumonite, et évidemment à toutes les complications parfois irréversibles que ces conditions peuvent provoquer.

Nous devons donc faire face à des complications infectieuses de manifestations allergiques extrinsèques, et nous devons également faire face à la conséquence inévitable de processus infectieux et bactériens prolongés, qui peuvent comme nous le savons tous, donner lieu à une sensibilisation bactérienne avec tout ce que cela peut comporter dans le domaine de l'allergie clinique.

Certaines caractéristiques du climat canadien donnent donc lieu chez-nous à une incidence extrêmement marquée de sensibilisation pollinique, et à des complications infectieuses qui font que chez-nous l'allergie extrinsèque doit être particulièrement respectée si nous voulons éviter qu'une bonne partie de notre population ne soit un jour handicapée par des lésions pulmonaires irréversibles.

AETIOLOGICAL FACTORS OF BRONCHIAL ASTHMA IN GREAT BRITAIN

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Allergens and respiratory infections can cause attacks of bronchial asthma based on a disturbance of immune mechanisms. The same syndrome, however, may be precipitated by psychological stimuli. In Great Britain an assessment of the relative importance of these three precipitating causes has been made by D. A. WILLIAMS and his co-workers at Cardiff, WALES (1958) and by R. S. BRUCE PEARSON in London (1958).

At Cardiff, in a study of 487 asthmatics, an infective factor was found to be present in 88 %, a psychological factor in 70 % and an allergic factor in 64 %. Rarely the asthmatic person responded to one cause only. Usually two or three causes were involved with one of them recognisable as the dominant factor.

Table I. 45 % of the 487 asthmatics gave evidence for one cause only. Thus the infective element solely was present in 34.7 %, the allergic cause in 10.7 % and the psychological in 1.2 %. The remaining 53.4 % were combined cases. A combination with psychological factors occurred in the same frequency in the infective and the allergic group, two thirds of the patients demonstrating this trend, though not neccessarily as a dominant cause.

Table II. When the dominant causes were considered it was noticed again the most common dominant factor was the infective. The surveys of 487 patients at Cardiff and 625 cases in London are well in agreement though investigated independently. 41 % and 42.3 % respectively were

TABLE I

Frequency of solely precipitating causes

	487 Patients Cardiff (*)
1. Infective	34.7 %
2. Allergic	10.7 %
3. Psychological	1.2 %

(*) D. A. WILLIAMS et al. (1958).

TABLE II

Frequency of dominant precipitating causes

	487 Patients Cardiff (*)	625 Patients London (**)
1. Infective	41 %	42.3 %
2. Allergic	29 %	33 %
3. Psychological	30 %	24.7 %

(*) D. A. WILLIAMS et al. (1958).
(**) R. S. BRUCE PEARSON (1958).

infective dominantly, 29 % and 33 % allergic and 30 % and 24.7 % respectively psychological.

Bronchial Asthma and bronchitis.—It can be concluded from the figures given above that in Great Britain the most common single factor leading to precipitation of attacks were respiratory infections, mainly bronchitis, also sinusitis. These infective episodes were very common in children below the age of 12 years, then they declined in frequency but occurred again more often in middle age and as a more serious factor. Bronchial asthma with bronchitis especially in age groups above 35 years was of greater severity, status asthmaticus occurred more frequently and the death rate was higher. The great severity of cases of bronchial asthma with bronchitis in higher age groups and often starting only in higher age groups was stressed also by J. L. LIVINGSTONE and J. PAGET-DAVIES (1961) and by A. G. OGILVIE (1962). The childhood bronchitis with asthma had a better prognosis than the bronchitis with asthma of the adult.

Table III compares the clinical severity with the incidence of the main precipitating factors in BRUCE PEARSON's study of 625 cases. Group I the most severe group, with incapacitating asthma, had the

TABLE III

Severity and precipitating factors

Severity	625 patients London (*)		
	Allergy factors	Infective factors	Emotional factors
Incapacitating	30 %	83.5 %	43.2 %
Severe	51 %	70.5 %	29.5 %
Moderate	76 %	61.0 %	22.0 %
Mild	72 %	44.7 %	19.2 %

(*) R. S. BRUCE PEARSON (1956).

highest incidence of infective factors, whereas group 3, a comparatively moderate group in which asthma could be controlled by simple means, had the highest incidence of allergic factors. This leads us to the conclusion that generally the allergens of Britain do not affect the asthmatic as badly as the bacteria, although in single cases the reverse may be true.

The importance of various climatic allergens in Great Britain, and the possible influence of air pollution on respiratory allergies in our big cities will be discussed.

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FRECUENCIA ETIOLOGICA DEL ASMA BRONQUIAL EN EL ECUADOR

G. MORENO VALERO (*)

Para poder explicar y comprender este aspecto de la patología alérgica en el Ecuador es preciso hacer conocer previamente la situación geográfica y climatológica del mismo, situado, como su nombre indica, bajo la línea ecuatorial.

Esta circunstancia y el hecho de que la cordillera de los Andes al atravesarlo de norte a sur divide al país en tres regiones geográficas que presentan cada una de ellas características propias, y son: el litoral o costa, que es la más extensa y está comprendida entre las estribaciones de los Andes y el Océano Pacífico; la región Interandina o Sierra comprendida entre los dos macizos de los Andes, interrumpidos de trecho en trecho por nudos de cordilleras secundarias que circunscriben las llamadas hoyas, y finalmente la región oriental o amazónica.

Por otro lado, en virtud de su situación francamente ecuatorial, el Ecuador presenta solamente dos períodos estacionales bien diferenciados: la estación seca, denominada verano, y la estación lluviosa o invierno. La primera comienza desde los primeros días de mayo hasta mediados de diciembre, y la segunda, el resto del año.

Realizadas estas consideraciones generales fundamentales, entraremos en materia para aportar nuestras observaciones referentes a los factores etiológicos del asma en el Ecuador, comenzando por conocer la frecuencia de esta enfermedad en el país en las dos principales regiones, tomando como centro principal de la costa a la ciudad de Guayaquil, donde confluyen las principales vías de comunicación de la región y donde tenemos nuestro lugar de actividades profesionales. En la región Interandina o sierra tomaremos como centro a la ciudad de Quito, capital de la república, que igualmente resume a toda la región.

De las observaciones realizadas hasta la fecha se ha podido determinar en forma bastante concluyente la existencia de zonas o regiones que presentan una incidencia muy alta de asma, en contraste con otras en las cuales es muy baja. Podríamos hablar con más propiedad de zonas asmógenas y no asmógenas.

A la primera categoría corresponde la región litoral o costa y principalmente la ciudad de Guayaquil, que es a la que nos vamos a referir en la presente comunicación.

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En esta zona distinguimos con marcadas características las dos estaciones que presentamos, iniciándose el verano o estación seca en los primeros días de mayo, coincidente con una baja apreciable de la temperatura, comprendida entre 20° a 30°, correspondiendo las cifras más bajas en las mañanas y en las noches, pero con un alto grado de humedad. Es la época de los fuertes vientos y días nublados y que facilita la presentación de cuadros gripales epidémicos, ostensiblemente manifiestos con el advenimiento de la estación que estamos describiendo. Es posible observar entonces un incremento de procesos infecciosos que asientan especialmente en las vías respiratorias altas con cuadros de rinitis, rinosinusitis, amigdalitis, adenoiditis, etc., cuadros que se presentan en adultos y niños de ambos sexos y se mantienen o repiten, pese a los tratamientos instaurados hasta la terminación de la estación. Esta localización alta de los procesos infecciosos constituye en muchos casos el comienzo de aquel síndrome ya conocido del catarro habitual descendente con su franca participación bronquial.

Especialmente hemos observado en niños desde pocos meses de edad la frecuencia de adenoiditis que han constituído el origen franco de comienzo de asma en un elevado porcentaje de casos.

Aparte de estos síndromes infecciosos, es una característica de la estación seca la presencia de fuertes vientos con mucho polvo en suspensión, cuya composición, de acuerdo a los estudios realizados sobre el mismo, lo constituye un porcentaje alto de bacterias diversas, hongos, epitelios de animales, pólenes, etc., que le comunican al mismo una intensa actividad alergénica que hemos comprobado a través de nuestras observaciones. En efecto en nuestras estadísticas encontramos que un porcentaje muy alto de pacientes con asma son altamente sensibles al polvo doméstico. Son pacientes que en su historia ya nos han relatado esta reacción, lo cual ha sido luego comprobado con los tests cutáneos respectivos, a la par que posteriormente con los tratamientos desensibilizantes y su respectiva evitación.

Merece también especial mención entre los factores etiológicos importantes en la zona que estamos describiendo, los hongos ambientales, cuya flora es muy rica y variada, circunstancia ésta que se ve favorecida por las condiciones ambientales. Lamentablemente no se han realizado estudios sobre los mismos de ninguna clase, y bástenos para comprender su enorme importancia que cualquier objeto doméstico que es guardado con humedad constituye un medio de cultivo ideal para estos hongos, que crecen de forma activa y es posible observarlos macroscópicamente a simple vista.

En escala menor tenemos pacientes que son sensibles a epitelios animales, inhalantes varios: lana, algodón, tabaco y en un porcentaje apreciable en recientes investigaciones en nuestro medio, a polvo de restos de insectos, cuya riqueza es extraordinaria en esta zona. También hemos encontrado algunos pacientes que responden a una etiología de pólenes diversos, aunque en este campo nuestra casuística es moderada, y por otro lado tampoco se han realizado estudios sobre nuestra flora anemófila y su relación estacional.

Hablando siempre de la región de la costa o litoral, en la estación de las lluvias o invierno, que comienza a fines de diciembre y se prolonga hasta los primeros días de mayo, es la época de temperaturas más calurosas, que van de 24° a 34° C., con torrenciales lluvias y un porcentaje permanente de humedad; igualmente es la época de la invasión de las plagas de insectos tropicales más variados.

En esta época del año, según nuestras observaciones, disminuyen los casos de asma, aunque existe la misma observación referente a los factores etiológicos, es decir, factores infecciosos, polvo doméstico, hongos, etc.

Es también en esta época que según nuestros estudios se acumulan grandes cantidades de hongos, así como también restos de insectos que al llegar la estación seca con sus vientos fuertes y el incremento de los procesos infecciosos, hacen de esta estación la predominantemente asmógena, factores a los cuales se añaden el tipo de vivienda nuestra, especialmente en las clases pobres, a base de madera y caña guadúa, fácilmente atacables por parásitos e insectos, así como también el uso muy difundido de colchones, almohadas y otros objetos rellenos de lana de ceibo, y en igual forma el uso de toldos o mosquiteros para protegerse de las plagas, especialmente en invierno, que constituye un medio ideal para retención de polvo con todas sus impurezas.

Resumiendo, en esta región de la costa ecuatoriana el asma bronquial es un problema médico que se presenta durante todo el año, pero con una mayor incidencia durante nuestro llamado verano o estación seca.

Las observaciones sobre este mismo problema en la región interandina o sierra las he tomado de los diversos trabajos realizados hasta la fecha por el doctor PLUTARCO NARANJO, cuyo centro de actividad lo constituye la ciudad de Quito, y que nos dice en términos generales que en esta región la incidencia de asma es muy baja, con excepción de ciertas zonas en las cuales pueden aplicarse las características descritas en la región de la costa y que constituyen zonas bajas y montañosas con sistemas hidrográficos que desembocan en la costa o en la región oriental.

Por lo demás, en esta zona predominan durante todo el año temperaturas frías, 6° y 8° como mínimas, hasta 18° a 20° C. como máximas. Goza igualmente de las dos estaciones ya descritas, pero con lluvias menos intensas y frecuentes y con un porcentaje de humedad más bajo que en la costa.

En lo que a los factores etiológicos del asma bronquial se refiere, en esta zona se observa un predominio de asmas de origen bacteriano, encontrándose igualmente un porcentaje muy alto de sensibilización a polvo doméstico, siguiendo luego pólenes en mayor proporción que en la costa, hongos diversos, inhalantes varios, restos de insectos en menor escala, etc. En lo que hace a alergenos alimenticios, nuestras estadísticas, tanto en la sierra como en la costa, son pobres en esta etiología, recordando que personalmente hacemos este tipo de investigación a base de dietas de eliminación y provocación sin emplear los tests cutáneos.

De la región oriental o amazónica no tenemos datos que reportar por carecer de profesionales dedicados a estos problemas en esas regiones de la patria.

Resumen y comentarios.

De lo expresado hasta aquí en la presente comunicación cabe hablar en el Ecuador de la existencia de dos zonas de incidencia del asma bronquial: la primera, la más importante, verdadera zona asmógena, que la constituye el litoral o costa, y la segunda, con bajo porcentaje, es la región interandina o sierra.

La estación con mayor incidencia la constituye en la región de la costa el verano o estación seca, aunque en general se presentan casos durante todo el año.

Que los factores etiológicos más frecuentes e importantes en las regiones principales del Ecuador son: factores infecciosos, polvo doméstico, hongos, inhalantes varios, pólenes, restos de insectos, etc., encontrando una pequeña diferencia en las zonas en lo que a los pólenes se refiere, que parecen tener mayor importancia en la sierra.

La alta capacidad alergénica que presenta nuestro polvo de casa, explicable tal vez por las condiciones de clima existentes.

La baja incidencia de asma producidas por alimentos y otros factores.

Que el asma bronquial constituye en el Ecuador un problema médico de mucha importancia y que lastimosamente no se le ha concedido la importancia que merece.

Que es necesario emprender nuevos estudios e iniciar otros que den más luz en el problema.

LA FRECUENCIA ETIOLOGICA DEL ASMA BRONQUIAL EN URUGUAY

J. A. MORETTI.

Antes de comenzar a desarrollar este relato, queremos felicitar a las autoridades del V Congreso Internacional de Alergología por haber elegido este tema, pues es en el asma bronquial, más que en cualquier otra afección alérgica, donde el factor regional tiene una real incidencia en su producción.

El presente trabajo es de orden y objetivación. Traducirá las conclusiones de muchos años de labor y trataremos de dar una visión lo más exacta posible del problema.

Creemos que es en las enfermedades alérgicas, más que en otras afecciones, sean éstas tóxicas, degenerativas o infecciosas, donde el factor geográfico tiene una importancia capital, ya que engloba con características propias a muchos de los más importantes elementos etiopatogénicos, sean ellos alimenticios, inhalantes, hongos, etc.

Este concepto y nuestro concepto sobre Alergia Regional, puede ser de valor como medio comparativo con lo que se observa en otras partes del mundo.

Cada región tiene sus características alergénicas, y es necesario conocerlas para poder valorarlas en su justo término y orientar así el tratamiento adecuado.

Conocemos bien la importancia del polen como agente etiológico del asma bronquial en los EE. UU. y fue con ese concepto que comenzamos a trabajar en los primeros años de nuestra especialidad, considerando en un orden secundario de importancia a los alergenos alimenticios. Pero no pasó mucho tiempo para que pudiéramos tener la primera constatación y una base fundada para pensar en los factores o condiciones regionales.

Y es ahora, después de muchos años de práctica en la especialidad, que se nos hace evidente que en nuestro país, sin lugar a dudas, son los factores alimenticios los que abarcan y condicionan la provocación del asma bronquial en la mayoría de los casos, no sólo en la primera infancia, sino también en la adolescencia y la edad adulta, junto con otros alergenos, sobre todo inhalantes, quedando en un segundo plano el factor polínico, y tanto es así, que sólo efectuamos la testificación con extractos de polen cuando la historia clínica así lo justifica.

De lo antedicho sacamos las siguientes conclusiones:

1) La alergia polínica es poco frecuente en nuestro país.
2) La alergia alimenticia es la primera sin lugar a dudas.
3) La alergia a inhalantes es muy digna de tenerse en cuenta.
4) La alergia a hongos debe ser valorada en cada caso en particular.
5) La alergia bacteriana debe ser encarada con criterio clínico.

La aseveración número 2 está sostenida por las siguientes razones: curaciones o mejoras muy importantes las hemos obtenido con tratamientos únicos o basados principalmente con regímenes alimenticios compuestos con los alimentos que dieron testificación intradérmica negativa, contemplando además cuidadosamente las intolerancias alimenticias manifestadas por los enfermos y orientados según indique el examen clínico en relación a su aparato digestivo, obesidad, diabetes, etc.

Podemos afirmar que los enfermos de asma bronquial, en un alto porcentaje de casos, sufren todo el año, sin o con pequeñas agravaciones estacionales y en muchas oportunidades tales agravaciones se producen en invierno, donde el factor polínico pierde todo su valor.

La frecuencia de los factores alergénicos alimenticos se debe, según nuestro criterio, a las siguientes causas no específicas, que bajan el umbral alergénico: 1) la diversidad de las preparaciones culinarias, donde se entremezclan cocina española, italiana, francesa, inglesa, etc.; 2) la cantidad de alimentos ingeridos, en muchos casos muy por encima del valor calórico requerido y con una desproporcionada cantidad de proteínas, sobre todo animales, por ser nuestro país eminentemente ganadero; 3) el hábito de no comer a hora fija, sino en cualquier momento, perturbando el ritmo digestivo; es muy corriente que se haga una sola comida, muy copiosa, en el almuerzo y en el resto del día sólo se come en forma muy irregular; 4) excesivo consumo de alcohol.

Nuestro trabajo está basado en el estudio estadístico exhaustivo de 169 casos de asma bronquial, seleccionados de más de 1.000 historias revisadas de nuestro Servicio de Alergia del Centro de Gastroenterología en el Hospital Pereira Rossell, dependiente del Ministerio de Salud Pública.

Todos los casos estudiados tienen su historia clínica completa, los exámenes de laboratorio necesarios y fueron seguidos en su curso clínico por un tiempo que excedió ampliamente el intervalo entre las crisis, pudiendo así valorar en su justo término el resultado del tratamiento, basado en la dieta antes descrita, indicaciones para inhalantes (eliminación o hiposensibilización), medicación adecuada dirigida a normalizar trastornos digestivos, cardíacos, etc. La medicación sintomática la utilizamos al principio del tratamiento etiológico, vigilando estrechamente a los enfermos en los que tenemos que utilizar los corticoides.

Registramos los siguientes datos estadísticos: número de ficha, nombre del enfermo, edad, nacionalidad, estado civil, fecha de consulta, antecedentes familiares alérgicos, antecedentes personales alérgicos, tiempo de enfermedad, intervalo entre las crisis, resultado de la testificación, análisis de laboratorio, tratamiento, resultado del tratamiento, tiempo de vigilancia.

DATOS ESTADÍSTICOS.

	Casos		Casos
Intervalo entre las crisis:		De 21 a 30 años	33
Diarias	65	De 31 a 40 años	23
De 1 a 10 días	26	De 41 a 50 años	21
De 11 a 15 días	14	De 51 a 60 años	10
De 16 a 30 días	20	De 61 a 70 años	7
De 1 a 2 meses	11	*Antecedentes familiares alérgicos:*	
De 3 a 4 meess	16	90 casos (52,66 %)	
De 5 a 6 meses	14	*Antecedentes personales alérgicos:*	
De 7 a 8 meses	2	46 casos (27,21 %)	
De 1 año	1	*Resultado del tratamiento:*	
Tipo de enfermedad:		0 %	35
Menos de 1 año	9	50 %	16
De 1 a 5 años	70	60 %	11
De 6 a 10 años	38	70 %	10
De 11 a 15 años	18	80 %	24
De 16 a 20 años	19	90 %	9
De 21 a 25 años	6	100 %	64
De 25 a 30 años	4	*Eritrosedimentación alta:*	
De 31 a 35 años	1	17 casos (10,05 %)	
De 36 a 40 años	2	*Parasitosis intestinal:*	
De 41 a 45 años	1	3 casos.	
De 56 a 60 años	1	*Enfisema pulmonar:*	
Edad:		5 casos.	
De 1 a 10 años	41	*Eosinofilia sanguínea:*	
De 11 a 20 años	34	De más del 5 %, 98 casos (57.09 %).	

Comentario de los datos estadísticos: en relación al *intervalo entre las crisis,* destacamos que de un total de 169 casos estudiados, 136 (80,47 por 100) no pasaba de dos meses, lo cual corrobora nuestra apreciación, que la gran mayoría de los asmáticos no tenían influencia estacional (polínica) evidente.

En relación al *tiempo de enfermedad,* 70 casos (41,42 por 100) oscilan entre uno y cinco años, y 38 casos (23,66 por 100), entre seis y diez años. En general, estos enfermos están en la etapa funcional del asma bronquial.

En relación a la *edad,* la mayoría de los casos de asma bronquial aparece antes de los treinta años.

En relación a los *antecedentes personales y familiares alérgicos,* que suman 136 casos (82,86 por 100), nos demuestra la importancia de estos antecedentes en los enfermos de asma bronquial.

En relación al *resultado del tratamiento,* 64 casos (37,86 por 100) curados y 43 casos (25,50 por 100) con una mejoría apreciable, que suman 107 casos (63,33 por 100), con resultado terapéutico excelente; 27 casos (15,97 por 100) con resultado terapéutico mediano y 35 casos (20,71 por 100) no curados.

Los siguientes *alimentos,* en escala descendente, según el estudio estadístico, predominaron en su acción alergénica: pescado, naranja, chocolate, huevo, leche, carne de cordero, cebolla, ajo, banana, tomate. En relación a los *inhalantes*: polvo de la casa, pelo de perro, tabaco, lana de oveja y pelitre. *Hongos*: penicillium, hormodendrum, alternaria, como los más importantes.

Factores no alergénicos: es común observar en nuestro país las bronquitis asmatiformes, que muchas veces son confundidas con el asma bronquial.

THE FREQUENCY OF ETIOLOGIC FACTORS IN BRONCHIAL ASTHMA IN JAPAN

K. NAKAMURA (*)

Before going into the subject of "the frequency of etiologic factors in bronchial asthma in Japan", I would like to explain briefly Japan's geographic position and the general pattern of living in Japan to make my speech more understandable to you.

Geographically, Japan, which, consists of four main islands, is located to the east of the Asian Continent in the northern hemisphere and its eastern coast faces the Pacific. It ranges between fourty-five degrees thirty minutes and thirty degrees of north latitude, and between one hundred and fourty-six degrees and one hundred and twenty-eight degrees of east longitude.

Meteorologically, it lies mostly in the temperate zone and has distinct seasonal changes of climate. The outstanding features of the climate are the rainy season in June and July, and the typhoon season in September and October. In addition, seasonal winds also have some effects on the climate of Japan. In Tokyo, the average temperature in February, the coldest month, is 6.1° C and in August, the hottest month, it is 29.6° C.

Topographically, sixty per cent of Japan is mountains or hilly regions, and open field suited for farming is rather limited.

From the cultural and industrial points of view, Japan has a population of about ninety-four millions. About twenty per cent are the farming population while the remainder are in other fields of industry, business and other professions. Recently there is a city-ward tendency of population and the high and rapid development of industries has brought forth new medical problems. Furthermore, with the changes of daily living conditions including the dietary life after the World War II, the incidence of diseases have also changed.

The history of research in allergy in Japan is short as compared with the other fields of medical science. Therefore, research on bronchial asthma, particularly from the etiological point of view, is unsatisfactory. I have been asked, however, to speak on the "frequency of etiologic factors of bronchial asthma in Japan", and therefore, I would like to present here a report based on some data submitted in Japanese journals.

The incidence of bronchial asthma in adults is about 1.2 per cent according to KAWAKAMI, who studied 494 adult subjects. In children, the percentage is 0.92 per cent in boys and 0.47 per cent in girls, making an average of 0.7 per cent, according to MITSUKAWA's study on 113,000 children in Tokyo.

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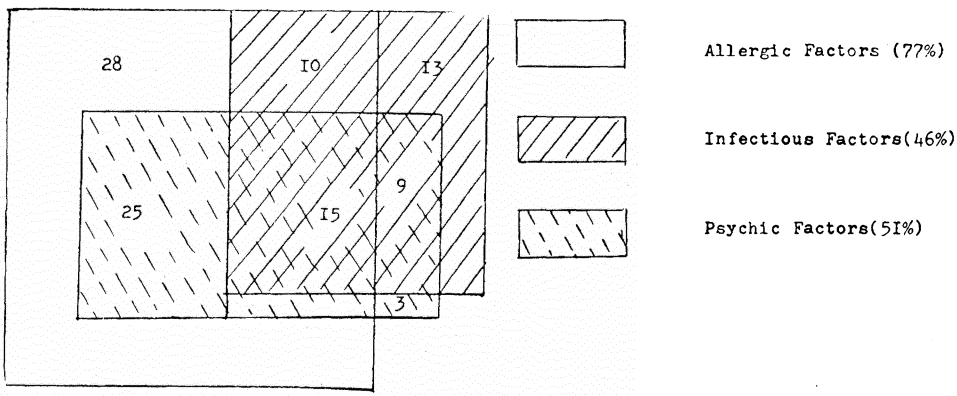
There are various methods for the etiological study of bronchial asthma. Allergic, infectious and psychological factors are considered to be the three major factors participating in bronchial asthma in Japan.

TABLE I

Etiologic Factors of Bronchial Asthma of Adults (Kobayashi)



KOBAYASHI studied 129 adult cases with bronchial asthma from these three aspects and reported the results as shown on Table I. It was found that allergic factor was participating in 77 per cent of the studied cases, infectious factor in 46 per cent and psychologic factor in 51 per cent, and there were a considerable number of cases where two or three factors acted together.

TABLE 2

Etiologic and Incentive Factors in the First Attack of Bronchial Asthma of Adults (MITSUI et al.)

	Cases	%
Unknown	231	34.7
Common Cold	229	34,4
Pneumonia	22	3.3
Measles	6	0.9
Whooping Cough	1	0.2
Bronchitis	17	2.6
Tuberculosis of Lung	6	0.9
Trauma	2	0.3
Inhalation of SO_2	2	0.3
Cold	10	1.5
Fatigue	13	2.0
Pregnancy	15	2.3
Psychic	1	0.2
Sea-squirt	80	12.0
Others	30	4.5
Total	665	100.0

Concerning the etiology and incentive factors of the 1st attack of bronchial asthma, MITSUI and his associates reported the results as shown on Table 2, based upon their studies on 665 adult asthma cases. Common cold was the predominant cause or incentive factor. Some other infectious diseases also were mentioned as the important factors. Though small in number, there were cases induced by SO_2 inhalation. 12.0 per cent were caused by sea-squirt, a kind of seaweed, which grows on the oyster shell. This is a local occupational asthma which is found in their research field, and about this I would like to speak again later. They failed to detect the etiological cause for 34.7 per cent of the studied cases.

TABLE 3

Etiologic and Incentive Factors in the First Attack of Bronchial Asthma of Children

(Research Group of Pediatric Allergy)

	Cases	%
Unknown	188	50.0
Common Cold	100	37.2
Pneumonia	12	3.2
Measles	10	2.7
Whooping Cough	8	2.1
Bronchitis	4	1.1
Typhoon	2	0.5
Infection	2	0.5
Dust	4	1.1
Cold	3	0.8
Fatigue	2	0.5
Psychic	1	0.3
Total	376	100.0

According to the Research Group of Pediatric Allergy, in 50 per cent of the 376 cases they studied, asthma developed without any specific cause. In 37.2 per cent it developed following common cold. In some cases, it followed pneumonia, measles, whooping cough or bronchitis as shown in Table 3.

Recently the skin test has become widely used to detect the allergen of bronchial asthma in Japan.

KAWAKAMI conducted skin tests for adults, using nine various inhalant antigens and obtained the results as shown in Table 4. The highest percentage of 61.2 proved positive with "house dust". Old cotton came next with 40.3 per cent, then Japanese "tatami" mat with 26.0 per cent and buckwheat chaff with 25.0 per cent. Buckwheat chaff is often used as stuffing for Japanese-style pillows and this is probably the reason

why positive incidence was so high with buckwheat. Ragweed was positive in only 10.7 per cent of the cases.

TABLE 4

Results of skin Test in Bronchial Asthma of Adults
Inhalants (KAWAKAMI et al.)

	Total	Positive	%
House Dust	397	243	61.2
Old Cotton	67	27	40.3
Mat (Japanese)	123	32	26.0
Buckwheat	108	27	25.0
Cat Hair	65	12	18.4
Dog Hair	66	9	13.6
Ragweed	75	8	10.7
Fungus (mixed)	140	97	69.3
Bacteria (mixed)	195	129	66.2

TABLE 5

Results of skin Test in Bronchial Asthma of Children
Inhalants (BABA et al.)

	Total	Positive	%
Wool	67	48	71.7
House Dust	229	141	61.6
Ragweed	212	71	33.5
Candida	181	54	29.8
Buckwheat Flour	193	48	24.9
Sandalwood	193	38	19.7
Old Mat	238	38	16.0
Rabbit Hair	200	31	15.5
Cladosporium	152	23	15.1
Cat Hair	189	28	14.8
Dog Hair	149	21	14.1
Feather	23	3	13.0
Cotton Wool	169	19	11.8
Alternaria	189	21	11.1
Silk	200	22	11.0
Ceder	184	19	10.3
Cotton	189	17	9.0
Tea Grounds	190	17	8.9
Penicillium	182	15	8.2
Aspergillus	185	15	8.1
Rice Straw	115	9	7.8
Pine	198	15	7.6
Hemp	184	12	6.5
Kapok	200	11	5.5
Chaffs	201	10	5.0
Japanese Cypress	134	5	3.7
Wheat Straw	89	2	2.2

BABA and his co-workers obtained these results from the skin tests for pediatric asthma. The highest incidence was obtained with wool but we have not yet ascertained the clinical significance of this fact. House dust, ragweed, candida and buckwheat flour appeared important in this order. It was quite interesting to find that the positive resuls with house dust were of high percentage in children as well as in adults. Ragweed had not been considered as an important allergen in Japan, but recently we came to experience occasional hay fever or bronchial asthma which is probably caused by ragweed.

TABLE 6

Results of Skin Test Using House Dust and Ragweed in Bronchial Asthma of Adults and Children

Reporter	House Dust Positive	Ragweed Positive
Kawakami et al.	61.2 %	10.7 %
Mitsui et al.	59.5 %	
Araki	67 %	33 %
Matsumura et al.	64 %	
Baba et al.	61.6 %	33.5 %

Here, I would like to show the results of skin tests with house dust and ragweed conducted by several other investigators. As understood from the table the incidence of positive reaction to "house dust" skin test was 60 to 70 por cent and in the case of ragweed, 10 to 30 per cent. At this point, it has to be emphasized that approximately half of the subjects who showed positive reaction to the skin test with house dust were also positive to the inhalant test with house dust.

TABLE 7

Results of Skin Test in Bronchial Asthma of Adults Foods (MITSUI et al.)

	Positive %
Milk	44.7
Cuttlefish	23.7
Buckwheat Flour	22.8
Bambooshoot	19.1
Whole Egg	13.6
Pork	12.7
Mackerel Pike	12.5
Mackerel	15.7
Flour	12.7
Bonito	10.0
Beef	7.3

MTSUI and his associates obtained the results as shown on Table 7, employing 11 food extracts.

TABLE 8

Results of Skin Test in Bronchial Asthma of Children
Foods (BABA et al.)

	Total	Positive	%
Yolk	29	7	24.1
Chocolate	20	4	20.0
Whole Egg	44	8	18.2
Milk	76	12	15.7
Chicken	20	3	15.0
Egg White	73	6	8.2
Mackerel Pike	25	2	8.0
Cuttlefish	36	2	5.6
Crab	37	2	5.4
Mackerel	37	2	5.4
Shrimp	41	2	4.9
Pork	66	3	4.5
Bonito	51	2	3.9
Beef	47	1	2.1
Spinach	58	0	0
Salmón	28	0	0
Eggplant	25	0	0

BABA obtained these results using 17 different food extracts. The significance of skin test as a means of detecting causative allergen in food allergy is still debatable.

TABLE 9

Relation between History of Allergy and Skin Reactions
in Bronchial Asthma of Adults
(KAWAKAMI et al.)

		History of Allergy		
		+	—	Total
Skin Reaction	+	100 (39.4 %)	67 (26.4 %)	167 (65.7 %)
	—	38 (15.0 %)	49 (19.3 %)	87 (34.3 %)
Total		138 (54.3 %)	116 (45.7 %)	254

254 adult asthma cases were divided into two groups, one group with past history or family history of allergy and another group without history of allergy, to study the relationship between the skin test results and the history of allergy. It was noted that 138 cases or 54.3 per cent had an allergic predisposition, and of the number 100 cases reacted to skin tests with one or more of inhalant allergens, whereas in the cases without allergic predisposition, only 49 cases or 19.3 per cent proved negative. In another word the incidence of positive reaction to skin tests was significantly higher in the group with allergic predisposition.

TABLE 10

Inheritance of Allergic Diseases in Children with Bronchial Asthma and in Control

(Research Group for Pediatric Allergy)

	Bronchial Asthma	Control
Bronchial Asthma	177 (47.1 %)	130 (20.4 %)
Other Major Allergy Manifestations	96 (25.5 %)	137 (22.5 %)
Negative	78 (27.1 %)	304 (54.6 %)
Unknown	1 (0.3 %)	16 (2.5 %)

In children,72.6 per cent of asthma cases were found to have allergic predisposition, while the rate was only 42.9 per cent in control.

It has also been reported that chemical substances such as histamine, choline and trimethylamine contained in food are responsible for asthmatic attack and such food is called pseudoallergen.

So far I have discussed the etiologic factors of bronchial asthma from the standpoint of antigen-antibody reaction. However, there are such investigators in Japan who are studying the etiology of bronchial asthma from the aspect of disorder of the autonomic nervous system. They presume that acetylcholine play a major role in the development of bronchial asthma. Some insist on the psychological factor. In fact, a considerable number of asthma cases can be explained on these theories.

Next, I would like to discuss the etiology of bronchial asthma in Japan from the meteorological standpoint. As I mentioned in the beginning Japan lies between the Asian Continent and the Pacific. Because of such a geographical position Japan has distinct seasonal changes in climate. For this reason, many investigators attach importance to meteorological changes as a possible cause of asthmatic attack. In the typhoon season of September and October the incidence of asthmatic

attack increases markedly, and it is a well known fact that some asthmatic patients can foretell the coming of a typhoon. In this connection, the relationship between the atmospheric condition around the Japanese islands and asthmatic attack has been reported by considerably many people, and most of them observe a close relation of the cold front with the incidence of asthmatic attack. On the other hand, NEMOTO notes a close correlation between the incidence of asthmatic attack and the moving high atmospheric pressure. He presumed that the inversion layer of temperature formed by the moving atmospheric pressure make allergens such as dust, pollen, mold, etc. float in the air, and he referred to the so-called Tokyo-Yokohama asthma as belonging to this type.

TABLE 11

Asthma Peculiar to Japan

Mabushi Asthma
Maiko (Konjac) Asthma
Buckwheat Flour Asthma
Kakinouchiko (sea-squirt) Asthma

Lastly, I would like to mention a few cases of bronchial asthma which are induced by allergens peculiar to Japan. They are *Mabushi asthma, Konjac asthma, Buckwheat flour asthma* and *Kakinouchiko asthma.* "Mabushi" asthma is induced when the patient is exposed to the odor of the secretion of silk worm. "Konjac" asthma is caused by inhalation of "maiko", the powder of Amorphophalus Konjac, which is flown off during the production of "Konnyaku", a jelly-like Japanese foodstuff, made from Amorphophalus Konjac. Buckwheat flour asthma develops when they eat buckwheat noodle which is most popular among the Japanese, or when they use such pillows that are stuffed with buckwheat chaffs. "Kakinouchiko" asthma is, as briefly mentioned before, a kind of local occupational allergy, and it is presumed that asthmatic attack occur by inhalation of seasquirt, a marine plant growing on the oyster shell, which is scattered in the air when the shell is cracked. Kakinouchiko in Japanese means those who are engaged in cracking the oyster shell by a hook to take out oyster.

I have outlined the etiological factors in bronchial asthma in Japan. Recently the interest in allergic diseases increased, and special emphasis is placed on the detection of the cause of bronchial asthma as the first step to medical treatment. I am looking forward to the future researches.

This report is a summing-up of the Japanase literatures in this field. Gratefully acknowledge my indebtedness to Dr. BABA for this.

BIBLIOTH
CATOLICA

THE AETIOLOGICAL FACTORS OF RESPIRATORY ALLERGY IN SOUTH AFRICA

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Under the term "Respiratory Allergy" are included conditions of the upper and lower respiratory tracts of the seasonal (hay-fever and seasonal asthma) and perennial (nasal and paranasal allergy and asthma) types. In view of the fact that respiratory allergy is not a notifiable disorder it is not possible to establish accurate statistical data in regard to its incidence as such or in its various forms. Under the circumstances the most that con be done, in spite of the lack of uniformity of observations and other disadvantages, is to consider patients who come to notice in one or more clinics and hospitals or under the care of a single or group medical practice. The conclusions come to in this paper are based upon personal interviews of some 5,000 patients of both sexes and including about 2,000 persons under 20 years of age suffering from some form of respiratory allergy over a 13-year period from 1951. These patients derived mainly from Johannesburg and districts but also from various parts of the country, are sent by their private doctors or from hospitals to the Allergy Department of this Institute for skin tests and recommendations regarding the approach to therapy on the basis of the clinical information supplied and from any evidence that may be obtained from the skin testing procedures. In addition to these, personal studies have been made of patients in other parts of South Africa which were visited at different times especially for the study of respiratory allergy in these regions. Apart from the clinical aspects mentioned large numbers of experimental studies have been carried out over the years of the atmospheric pollens and fungi, climate circumstances, occupational and domestic contacts in homes and farms, etc. in relation to respiratory allergy in the various parts of the country.

Seasonal Respiratory Allergy

a) *Pollens.*

In South Africa seasonal respiratory allergy [1] is almost entirely confined to the summer months—October to March—with a maximum incidence in January and February. It is in this period that the Grasses

(*Graminae* spp.) are flowering and the pollens of the Grasses are the agents responsible for this summer hay-fever. It is interesting to note that the Grasses are not uniformly distributed throughout South Africa but grow in relatively well-defined regions in great abundance more especially in the Highveld, a high-altitude area occupying the major portion of the interior in the north easterly part of the country. The remaining regions of South Africa of lower altitudes right down to sea-level have far less grass and sufferers generally derive benefit when they leave the Highveld during the summer for these other regions and particularly the coast which is virtually devoid of grass. Clinically seasonal grass pollen respiratory allergy is manifested mainly as hay-fever and often accompanied by asthma. It is exceptional for true allergic asthma to occur alone as a seasonal condition without accompanying upper respiratory manifestations.

In the spring period of the year—the latter part of August, and September and October—both upper and lower respiratory allergy symptoms occur as a result of the inhalation of the pollens of the principal spring-flowering trees which are Poplar (*Populus* spp.), Oak (*Quercus* spp.) and Plane (*Platanus* spp.). The Prosopis tree [2] (*Prosopis* spp.) flowers in October and November and its pollen is sometimes a cause of respiratory allergy in parts of South Africa where this tree is found, but relatively frenquently in South West Africa where Prosopis trees are more abundantly present in the various towns.

Respiratory allergy occurring in the winter months has been recognised as due to the inhalation of the pollen of the Cypress tree [3] (*Cupressus* spp.) which is present in the atmosphere from May until October.

It is of interest to observe that in South Africa the pollens of the Grasses and the Trees are the only significant factors in seasonal respiratory allergy. Pollinosis due to Weeds is not a problem as it is in the United States of America and in certain parts of Europe where weed pollens give rise to respiratory allergy in the autumn months. Persons are occasionally affected by the pollens of Khaki weed (*Tagetes minuta*) and Cosmos (*Cosmos bipinnata*)—plants of the Compositae family—which grow mostly in waste lands. They generally make their appearance in the late summer—February to April. These plants however are rapidly disappearing near cities in face of the advance of the building areas and in the rural areas in farming districts they are of little importance because of the small and scattered population there.

b) *Atmospheric Fungi.*

Contrary to reported experience overseas the atmospheric fungi are only occasionally of clinical significance in South Africa [4] although a large proportion of patients with respiratory allergy show positive skin reactions to the commoner atmospheric fungi which include in order of relative abundance (approximately 30 % — 5 %) : Cladosporium, Yeast, Epicoccum, Penicillium, Alternaria, Pullularia and Phoma [5]. There is no significant seasonal incidence of these in the atmosphere except in the Yeast organism (yeasts, monilia, torula, etc.) where a consistent increase

occurs each year in the period May to October. Slide-exposure studies have revealed an increased seasonal incidence of Smuts in the early summer (October to December). Correlation of these increases cannot however be demonstrated with exacerbation of clinical symptoms.

c) *Other.*

Asthma does occur in the summer as an occupational disease in persons employed at or living in the vicinity of certain sewage disposal works [6]. The asthma, generally with upper respiratory symptoms is due to the inhalation of the fine dust resulting from the disintegration of the masses of accumulated bodies of the sewage flies (*Psychoda* spp.) which breed at the sewage works in the summer. Desensitization with extracts of *Psychoda* has proved successful in these cases.

Perennial (Non-Seasonal) Respiratory Allergy.

Specific animal hair, feather, house dust and foods as well as stress factors occur as the aetiological or precipitating agents of respiratory allergy in South Africa as they do elsewhere and thus do not merit special consideration here. House dust sensitivity however has been shown to be of special interest and is discussed in association with climate.

Climate.

The significance of "climate" as a factor in the occurrence of respiratory allergy in South Africa has been investigated for many years [7, 8, 9, 10]. One of the most interesting aspects of perennial allergy is associated with the patients who maintain relatively good health in the inland parts of the country but who develop asthma when they live at or even visit the coastal regions, particularly the east coast. We have referred to these as the "Climate Group" of sufferers, on the supposition that the markedly different climate patterns in these two regions explained the precipitation of symptoms at the coast. At the coast the temperature and relative humidity are both high and within narrow range during the 24 hours of the day and throughout the year. In contrast to this, the climate pattern of the inland areas reveals a lower temperature and relative humidity but, more important, there is a wide range of these two factors in the 24 hours and throughout the year. During the considerable studies that have been carried out it was shown that neither fungi nor pollens or other exogenous agents could be incriminated. With the passing of time and with much experience of large numbers of patients belonging to this "Climate Group" the concept of climate *per se* as a factor in the occurrence of symptoms at the coast became less significant. Indeed, it is now our belief that there is probably no *direct* effect of climate in the precipitation or even the exacerbation of symptoms. In the same way that climate is significant in pollen allergy as an indirect factor in the regulation of the type of plants that will grow under certain climate circumstances, so in the "Climate Group"

of patients it is our opinion that climate acts *indirectly* by influencing in some fashion an agent or agents which may be the triggering mechanism for symptoms at the coast. The most striking finding that emerged during the skin testing of patients belonging to the "Climate Group" was that they invariably showed a marked reactivity to House dust. As a result of this finding further experiments were carried out and it was demonstrated that the House dust obtained from the coast was allergenically more potent than that obtained from inland regions. The cause of this difference in potency was at first considered to be due to the effect on the dust at the coast of fungi and bacteria prevalent in these more humid regions. This hypothesis however was discarded when it was shown that the effect of such biological action was a reduction and not an enhancement of potency. Our present hypothesis which is being closely studied is that coastal House dust is in fact, "normal". In inland regions however in the presence of much brilliant sunshine and thus maximum ultraviolet radiations and, in the absence of humidity with its attendant mists and the smoke and smog associated with shipping and industries at the coast, there is a reduction of the potency of the local House dust. This concept is being put to the test by submitting coastal House dust to ultraviolet light, direct sunlight and other means to find whether the allergenic potency is reduced under such influences.

Cereals.

Large portions of South Africa are important agricultural areas and an extensive acreage is devoted in certain districts to the growing of cereals with the *Graminae;* Maize (*Zea mays*) and Wheat (*Triticum* spp.) constituting the largest crops. Other cereals such as Kaffir-corn (*Sorghum vulgare*), Rye (*Secale cereale*), Oats (*Avena sativa*), Barley (*Hordeum vulgare*) and Rice (*Oryza sativa*) are extensively grown. In addition however, Lucerne *(Medicago sativa)* is planted to a very considerable extent. Respiratory allergy including asthma is not infrequently found in persons who handle the grain [11], [12] either during the process of storing or milling and so inhale the dusts of the dry cereals or lucerne. These persons include farmers, millers and workers in grain stores. Symptoms occur also in bakers and housewives who handle the flour when baking. Desensitization with the extracts of the specific grain dusts has been found effective.

During the war period when wheat was in short supply in South Africa and flour substitutes were used in the baking industry a number of cases of asthma occurred in persons hypersensitive to Buckwheat [13] (*Fagopyrum vulgare*) both by inhalation and by ingestion.

Woods.

Cases of asthma have been investigated which were associated with the inhalation of Wood dust [14], [15] by persons working with various woods in their occupation as carpenters or cabinet-makers. The woods involved included Kejaat (*Pterocarpus angolensis*), Western Red Cedar (*Thuja*

plicata) and certain Congo "hardwoods". Desensitization with the specific wood extract was successful.

Castor Seeds.

The Castor oil plant (*Ricinus communis*) is being cultivated in certain parts of the country to an increasing extent and upper respiratory allergic symptoms as well as asthma occur as a result of the inhalation of the dust of the crushed beans or seeds in sensitive persons who handle this material [16], [17].

Other.

Respiratory allergy associated with foodstuffs occurs as indeed it does elsewhere and is largely found in children. Trial diets are resorted to in order to establish the responsible agents-commonly milk, eggs or cereals. Asthma from animal contact is sometimes seen more especially at home from dogs or cats or in the open from horses during horse-riding or visits to stables. Respiratory allergy attacks have been reported mainly in children during or following upon visits to the circus or agricultural shows where horses and cattle are present. Occasional cases of asthma occur in farmers and others when they handle and so inhale the dust from cattle and poultry feeds. It is interesting to note that respiratory allergy has been found to be associated with the drinking of "soft drinks" largely of the bottled type of synthetic fruit juices and other drinks most of which are associated with a preservative. Avoidance of these drinks has led to the amelioration or even the cessation of the respiratory allergy symptoms.

Racial Distribution.

The whole problem of the incidence of respiratory allergy in the different race groups is under investigation and it is hoped that more precise data will in due course become available. The subject is of the greatest interest and we have conducted numerous studies at different times and in various parts of the country [18]. But the pattern is a complicated one not only because of the language difficulties often involved in communication with certain sections of the indigenous Non-White population but also because of their regional, rural or urban distribution with the associated differences in type of work and foodstuffs consumed. Broadly, however, if the incidence of respiratory allergy in the White or European population is taken as the standard then the "Coloured" or Eurafrican section among the Non-Whites can be considered as having approximately the same incidence. In the indigenous Bantu peoples the incidence of asthma is relatively small. This applies to our experience with the urban native peoples of the inland Highveld regions of the country. Reports from the coastal area of the south western Cape would indicate a relatively higher incidence. On the other hand, amongst the Non-White inhabitants of the country the incidence of respiratory

allergy is relatively high in the Indian population largely occupying the coastal and inland areas of Natal and to a lesser degree also the Transvaal. The reason for this higher incidence in the Indians may be associated with the greater emotional instability in these people. This question is under investigation as is also the possibility that some of the foodstuffs peculiar to the diet of the Indians may contribute to this incidence.

SUMMARY.

An account is given of the principal aetiological factors in respiratory allergy in South Africa as observed over a 13-year period in some 5,000 patients of both sexes and including about 2,000 persons under the age of 20 years.

It is shown that Grass (*Graminae*) pollen is the principal factor in summer respiratory allergy in the interior highlands of the country where grasses occur in abundance. The pollens of the trees Plane (*Platanus* spp), Poplar (*Populus* spp.) and Oak (*Quercus* spp.) which flower in September and October give rise to pollinosis in the spring. The Cypress tree (*Cupressus* spp.) and the Prosopis (*Prosopis* spp.) are the agents of pollinosis in winter and early summer respectively. It is emphasized that contrary to experience in the Northern hemisphere weed pollen allergy virtually does not exist.

Atmospheric fungi are not considered to be of significance in the causation of respiratory allergy despite the frequency of positive skin reactions thereto. Reference is made to asthma and upper respiratory allergic symptoms aetiologically associated with the flies (*Psychoda* spp.) occurring in abundance during the summer at various sewage disposal plants.

The significance of the influence of climate is discussed and comment made on the part played by house dust in this regard.

In non-seasonal respiratory allergy the aetiological importance is mentioned of the dusts of grain (cereals, lucerne, etc.), wood and Castor Seeds by inhalation. Reference is made to the racial incidence of respiratory allergy in the various population groups of the country-European and Non-Whites including the indigenous Bantu, the Eurafrican and the Indian sections of the population.

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FACTORES ETIOLOGICOS DEL ASMA BRONQUIAL EN VENEZUELA

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La información disponible sobre esta afección la hemos dividido en dos grandes grupos: un grupo perteneciente a la infancia y otro a la edad adulta.

ASMA INFANTIL.

En el IV Congreso Panamericano de Pediatría, realizado del 7 al 13 de agosto de 1960, presentamos nuestras observaciones sobre 192 niños afectos de asma bronquial, cuyas edades oscilaban de menos de un año a los catorce años; en la edad pre-escolar encontramos 106 casos, o sea 55,2 por 100; en la edad escolar, en 82 casos, o sea 42,7 por 100. De estos niños 118 eran de sexo masculino, o sea el 62,8 por 100, y 70 eran de sexo femenino, o sea el 37,2 por 100.

Herencia.

El análisis de los antecedentes hereditarios puso en evidencia la existencia de 37 casos sin antecedentes hereditarios de alergia, o sea 19,3 por 100, y 155 casos con antecedentes hereditarios de alergia, o sea el 80,7 por 100. Pudimos comprobar que en 146 casos no existían antecedentes de alergia anteriores, o sea en el 76 por 100 de los casos; en cambio, 46 casos, o sea el 24 por 100, tenían antecedentes alérgicos en orden de importancia fueron: eczema, 17 casos; urticaria, 16, y rinitis, 12.

Asociaciones.

En el estudio de las manifestaciones alérgicas asociadas encontramos que 104 casos, o sea el 54,2 por 100, no presentaban manifestaciones de alergia asociada; en cambio, en 88 casos, o sea el 45,8 por 100, encontramos otras manifestaciones alérgicas asociadas al asma bronquial,

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entre las cuales merecen especial atención la rinitis en 49 casos, o sea el 25,5 por 100; la rinitis asociada a otras manifestaciones alérgicas no asmáticas en 25 casos, o sea el 13 por 100.

Sensibilización clínica: inhalantes.

En estos niños pudimos comprobar los alergenos más frecuentemente incriminados como responsables del asma bronquial que sufrían; eran en orden de frecuencia dentro del grupo de los inhalantes: primero, el polvo doméstico, cuya sensibilización la pudimos comprobar en el 34,4 por 100 de los casos, siguiéndole en orden de importancia los hongos saprofitos de la atmósfera con el 23,4 por 100 de los casos; en tercer lugar, las plumas, con el 3,6 por 100 de los casos; en cuarto lugar, los pólenes, con el 2,6 por 100 de los casos, y en el quinto lugar, la lana y el algodón, con 2,1 por 100.

Insectos.

La sensibilización de los insectos a través del resultado de las pruebas cutáneas practicadas con resultados positivos de intradermo-reacciones en 94 casos, puso en evidencia que fueron más frecuentes en el orden siguiente: mosca casera, en el 42,6 por 100 de los casos; escarabajo y abeja, en el 26,6 por 100 de los casos; saltamonte, en el 25,5 por 100 de los casos; mariposa, en el 24,5 por 100; polilla, en el 22,3 por 100 de los casos; avispa, en el 21,3 por 100 de los casos; abejorro, en el 20,2 por 100 de los casos, y el pulgón, en el 17 por 100 de los casos.

Alimentos.

Un estudio sobre las principales sensibilizaciones de tipo alimentario puso en evidencia que los alergenos de este tipo más frecuentemente encontrados fueron: el cacao y sus derivados, en el 7,3 por 100 de los casos; la leche, en el 2,6 por 100 de los casos; el huevo y las caraotas negras o frijoles negros, en el 2,1 por 100 de los casos; la naranja, en el 1,6 por 100 de los casos; el maíz y el banano, en el 1 por 100 de los casos.

Resumen.

Concluimos que en este grupo había un predominio de asma en los varones sobre las hembras; que los factores hereditarios influenciaban parcialmente como factores predisponentes en el desarrollo del asma; que en el 25 por 100 de los casos estudiados presentaron otras manifestaciones alérgicas previa a la aparición del asma.

Que en el asma infantil tienen los inhalantes una significación importante, esencialmente el polvo doméstico, las esporas de los hongos saprofitos de la atmósfera y las emanaciones de insectos.

Que los alimentos de mayor sensibilidad desde el punto de vista de interrogatorio son: el chocolate y el cambur; y que las pruebas cutáneas

muestran mayor sensibilidad por las caraotas negras, el trigo, la carne de res, leche, naranja y huevo; por tanto hay que ser cuidadoso en la valoración de estos resultados. Que las infecciones respiratorias altas tienen un papel determinante en la producción del asma bronquial en el 14,6 por 100 de los casos en las mismas.

Que los enfriamientos y trastornos emocionales actúan como factores agravantes y desencadenantes del asma bronquial.

ASMA EN LA EDAD ADULTA: CARACTERÍSTICAS DEL GRUPO

La segunda parte del presente estudio está constituída por nuestras observaciones sobre 300 casos de asma bronquial en adultos, de los cuales 192 eran mujeres y 108 hombres, o sea que el 64 por 100 de los casos estaban representados por individuos del sexo femenino, y el resto, o sea el 36,34 por 100, por individuos del sexo masculino.

Edad.

La distribución por edades de la muestra estudiada da la siguiente demostración: de catorce a veinte años, 58 casos, o sea el 19,33 por 100, veintiuno a treinta años, 81 casos, o sea el 27 por 100; de treinta y uno a cuarenta años, 60 casos, o sea el 20 por 100; de cuarenta y uno a cincuenta años, 36 casos, o sea el 12 por 100; de cincuenta y uno a sesenta años, 36 casos, o sea el 12 por 100, y por encima de los sesenta años, 29 casos, o sea el 9,677 por 100. Como se pudo apreciar en esta demostración, el asma bronquial en la muestra de adultos estudiada presenta una mayor incidencia entre los catorce y cuarenta años, o sea en las etapas de la vida en que el ser humano desarrolla su máxima capacidad física y reproductiva, predominando ampliamente el sexo femenino sobre el masculino.

Herencia.

La existencia de una tendencia hereditaria para adquirir esa enfermedad se observó en 136 casos, o sea en el 45,3 por 100 de ellos, y no fue referida en 163 casos, o sea en el 54,67 por 100 de ellos, de lo cual deducimos que en la muestra examinada predominan las manifestaciones de asma bronquial de tipo atópico.

Antecedentes personales alérgicos.

Entre las manifestaciones alérgicas previas merecen especial mención la existencia de 31 casos de rinitis, o sea 10,33 por 100, y de 11 manifestaciones de otro tipo, no rinítico, precediendo a la aparición del asma bronquial, o sea el 3,66 por 100.

En 187 casos, o sea en el 65,66 por 100 de ellos, pudimos determinar la influencia del ambiente del hogar en la aparición de las crisis asmáticas que se presentaron en 38 de ellos, o sea en el 16 por 100 de los casos dentro del hogar y el resto fuera de él.

Fuentes de alergenos más comunes en el hogar.

A fin de establecer las fuentes de alergenos más comunes en el domicilio de los pacientes asmáticos estudiados en esta prueba, se realizó una encuesta que demostró que de 205 pacientes 144, o sea el 70,24 por 100, vivían en casas nuevas y 81, o sea el 29,76, en casas viejas que tenían más de cinco años construidas. En las casas nuevas el techo era de concreto en 100 ellas, o sea en el 69,44 por 100 de los casos; que era de tejas en 11 casos, o sea en el 7,63 por 100; de cinc en 4 casos, o sea en el 2,77 por 100; 24 casos de un material no especificado, o sea el 16,66 por 100. En las casas viejas el techo era de tejas en 40 casos, o sea en el 49,38 por 100; en 8 casos era de concreto, o sea en el 9,87 por 100; en 7 casos era de asbesto, o sea en el 8,64 por 100; en 5 casos era de cinc, o sea en el 6,17 por 100, y en 20 casos no fue especificado, o sea en el 24,69 por 100.

El tipo de colchón más frecuentemente utilizado fue el de algodón en 225 casos, o sea en el 75 por 100; siguiéndole el de espuma de goma en 18 casos, o sea en el 6 por 100, y el de plumas en 14 casos, o sea en el 4,66 por 100.

El tipo de frazada más frecuentemente utilizado fue la de algodón en 138 casos, o sea en el 46 por 100, siguiéndole la de lana en 110 casos, o sea el 36,66 por 100 de los casos.

El material empleado en las almohadas de estos pacientes fue la espuma de goma en 91 casos, o sea en el 30,33 por 100 de ellos; el algodón en 75, o sea en el 25 por 100; el kapok en 33 casos, o sea en el 11 por 100, y la lana en 21 casos, o sea en el 7 por 100.

El material empleado para los muebles del domicilio más frecuentemente utilizado fue de madera en 236 casos, o sea en el 78,66 por 100; asociado al algodón en 62 casos, o sea en el 20,66 por 100, y el hierro en 15 casos, o sea en el 5 por 100. El hierro fue empleado en 30 casos, o sea en el 10 por 100, y el junco en 12 casos, o sea en el 4 por 100.

Las cortinas más frecuentemente ultilizadas fueron las de algodón en 52 casos, o sea el 17,33 por 100; la de seda en 29 casos, o sea en el 9,66 por 100.

El tipo de material más frecuentemente ultilizado en las alfombras por estos pacientes fue la de lana en 21 casos, o sea el 7 por 100, y el algodón en 10 casos, o sea el 3,33 por 100.

El tipo de material más frecuentemente utilizado para la confección de los vestidos por estos pacientes fue el algodón en 230 casos, o sea en el 76,66 por 100; el algodón asociado a la lana y la seda en 94 casos, o sea en el 31,33 por 100; a la lana en 47 casos, o sea en el 15,66 por 100, y en la seda en 16 casos, o sea en el 5,33 por 100.

Un estudio de los animales domésticos que se encuentran en el ambiente domiciliario de estos pacientes reveló que el animal más frecuentemente encontrado fue el perro en 49 casos, o sea en el 16,33 por 100; en convivencia con los gatos en 17 casos, o sea en el 5,66 por 100. En los gatos aisladamente en 34 casos, o sea en el 14,66 por 100; los pájaros en 34 casos, o sea en el 11,33 por 100, y por último las gallinas en 22 casos, o sea en el 7,33 por 100.

Clima.

En cuanto a la influencia de las diferentes épocas del año sobre la aparición de las crisis asmáticas en el grupo estudiado, pudimos comprobar que las crisis eran más frecuentes en los meses de mayo en 124 casos, o sea en un 41,33 por 100; en junio en 62 casos, o sea en 20,66 por 100; en agosto 15 casos, o sea un 5 por 100; en febrero en 14 casos, o sea en un 4,66 por 100; en septiembre y octubre en 9 casos, o sea en el 3 por 100, y en marzo con 4 casos, o sea en el 1,33 por 100; o sea que la mayor frecuencia de crisis se registra en los meses que coinciden con el comienzo de nuestra estación lluviosa, donde se registran las más elevadas temperaturas y humedades relativas del año, sobre lo cual debe influir evidentemente la proliferación de los hongos saprofitos de la atmósfera que se observan en estas condiciones y que constituyen una de las principales causas de asma en el grupo estudiado por nosotros.

Asociaciones.

El asma bronquial del adulto la encontramos asociada a la rinitis en 201 casos, o sea en el 67 por 100 de ellos; a la conjuntivitis en 89 casos, o sea en el 29,66 por 100, y al eczema en 27 casos, o sea en el 9 por 100.

Infecciones.

La infección crónica de las vías respiratorias altas (anillo de Waldeyer) la pudimos constatar en 62 casos estudiados, o sea en el 20,66 por 100.

Sensibilidad clínica para las drogas.

Pudimos comprobar sensibilización clínica para el uso de la penicilina en 22 casos, o sea en el 7,33 por 100 de ellos; sensibilización a la aspirina en 10 casos, o sea en 3,33 por 100; el yodo y sus derivados en 10 casos, o sea en el 3,33 por 100; a la efedrina en 11 casos, o sea en el 3,66 por 100, y a la tetraciclina en 5 casos, o sea en el 1,66 por 100 de ellos.

Sensibilidad clínica para los inhalantes.

En el grupo de los alergenos inhalantes pudimos comprobar por la anamnesis sensibilización para el polvo doméstico en 161 casos, o sea en el 53,66 por 100 de ellos; para los hongos saprofitos de la atmósfera en 150 casos, o sea en el 50 por 100 de ellos; para la lana en 63 casos, o sea en el 21 por 100 de ellos; para el lirio de florencia en 59 casos, o sea en el 19,66 por 100; para las plumas en 47 casos, o sea en el 15,66 por 100; para el algodón 32 casos, o sea en el 11,66 por 100. Entre las asociaciones de sensibilizaciones para inhalantes obtenidas por la anamnesis del paciente merecen destacarse la del polvo con el piretro y los hongos saprofitos de la atmósfera en 73 casos, o sea el 24,33 por 100; la del polvo con los hongos saprofitos en 53 casos, o sea en el 17,66 por 100; la del polvo y el piretro en 18 casos, o sea en el 6 por 100 de ellos.

La lana la encontramos asociada a la epidermis del perro en 9 casos, o sea en el 3 por 100; a la del perro y el gato en 9 casos, o sea el 3 por 100, y a la del gato solamente en 5 casos, o sea en el 1,66 por 100 de ellos. La sensibilidad al lirio de florencia la encontramos asociada al algodón y las plumas en 10 casos, o sea el 3,33 por 100, y en casos el algodón, o sea en el 1 por 100. Las plumas las encontramos asociadas al algodón en 9 casos, o sea en el 3 por 100 de ellos.

Sensibilidad clínica para los alimentos.

A través de la anamnesis encontramos que las sensibilizaciones alimentarias más frecuentes fueron: el chocolate en 24 casos, o sea en el 8 por 100; los huevos en 21 casos, o sea en el 7 por 100; el pescado en 14 casos, o sea en el 4,66 por 100; el pollo en 12 casos, o sea en el 4 por 100; el banano en 12 casos, o sea en el 4 por 100; las caraotas negras o frijoles en 21 casos, o sea en el 7 por 100; los camarones y langosta en 10 casos, o sea en el 3,33 por 100, y el trigo en 12 casos,o sea en el 4 por 100. Para la leche en 8 casos, o sea en el 2,66 por 100.Las asociaciones alimentarias más frecuentes encontradas a través del interrogatorio fueron: la del huevo y la leche en 6 casos, o sea el 2 por 100; la leche y el pescado en 3 casos, o sea en el 1 por 100; la del pollo y el banano en 3 casos, o sea en el 1 por 100; la del trigo y las caraotas negras o frijoles en 3 casos, o sea en el 1 por 100.

Pruebas de eliminación y exposición.

Se estudió la sensibilización alimentaria en 126 casos por las dietas de eliminación y exposición, encontrando sensibilización para el pescado en 30 casos, o sea el 23,80 por 100; para los huevos en 30 casos, o sea el 23,80 por 100; para la leche en 29 casos, o sea 23,01 por 100; para el trigo en 19 casos, o sea 15,07 por 100.

Pruebas cutáneas.

El estudio de los alergenos inhalantes más frecuentes en nuestro medio con intradermo-reacciones al título de 1 por 100.000 dieron resultado positivo para el polvo doméstico en 226 casos, o sea en el 75,33 por 100; asociado a los hongos saprofitos de la atmósfera en 147 casos, o sea en el 49 por 100.

Los hongos saprofitos de la atmósfera dieron resultado positivo en 216 casos, o sea en el 72 por 100. El lirio de florencia resultó positivo en 52 casos, o sea en el 17,33 por 100, y asociado al polvo doméstico en 9 casos, o sea en el 3 por 100; el piretro dio resultado positivo en 28 casos, o sea en el 9,33 por 100; asociada al kapok en 8 casos, o sea en el 2,66 por 100. El kapok dio resultado positivo en 33 casos, o sea en el 11 por 100; asociado al piretro en 8 casos, o sea en el 2,66 por 100, y al tabaco en 5 casos, o sea en el 1,66 por 100.

El tabaco dio resultado en 26 casos, o sea en el 8,66 por 100.

El papel periódico dio positivo en 34 casos, o sea en el 11,3 por 100 asociado al algodón en 11 casos, o sea en el 3,66 por 100 de los casos.

Un estudio realizado a través de la intradermo-reacción de pólenes más frecuentes de la localidad dio su mayor incidencia de pruebas cutáneas positivos con la grama (Cynodon dactilus) en 37 casos, o sea en el 12,33 por 100; asociado al polen de coco (Coccus plumosa) en 7 casos, o sea en el 2,33 por 100. El polen del tártago (Ricinus comunis) dio resultado positivo en 35 casos, o sea en el 11,66 por 100; asociado al pazote (Quenopodium ambrosioides) en 7 casos, o sea en el 2,33 por 100. Luego le sigue en importancia el pazote (Quenopodium ambrosioides) con 24 casos, o sea en el 8 por 100; asociado al tártago en 7 casos, o sea en el 2,33 por 100.

Entre los pólenes de árboles merecen destacarse los procedentes del cedro en 25 casos, o sea en el 8,33 por 100; el del eucalipto en 19 casos, o sea en el 6,33 por 100, y el del pino en 18 casos, o sea en el 6 por 100.

El estudio de la epidermis de animales domésticos más comunes a través de los resultados de las intradermo-reacciones reveló sensibilidad para la lana en 42 intradermo-reacciones, o sea en el 14 por 100; para el perro en 37 casos, o sea el 12,33 por 100; para el ganado vacuno y el gato en 28 casos, o sea en el 9,33 por 100; para el zorro en 21 casos, o sea en el 7 por 100; para las plumas en 21 casos, o sea en el 7 por 100, y para el conejo en 19 casos, o sea en el 6,33 por 100.

Las intradermo-reacciones practicadas con las emanaciones de insectos pusieron en evidencia sensibilidad cutánea positiva en 93 casos para la abeja, o sea en el 31 por 100; para el mosquito en 81 casos, o sea en el 27 por 100; para la mosca doméstica en 79 casos, o sea en el 26,33 por 100; para la seda en 77 casos, o sea en el 25,66 por 100; para la mariposa en 69 casos, o sea en el 23 por 100; para la polilla en 64 casos, o sea en el 21,33 por 100; para el abejorro en 60 casos, o sea en el 20 por 100; para la avispa en 51 casos, o sea en el 17 por 100. Como asociaciones interesantes merecen referirse las de la abeja y la seda con el mosquito en 18 casos, o sea el 6 por 100; la del mosquito con la abeja en 16 casos, o sea en el 5,33 por 100; la del mosquito con la seda en 15 casos, o sea el 5 por 100; la de la mosca con la polilla en 21 casos, o sea el 7 por 100; la de la mosca con la mariposa en 19 casos, o sea el 6,33 por 100, y la asociación de la polilla con la mosca en 21 casos, o sea en el 7 por 100.

Resumen.

En la muestra estudiada hay franco predominio del asma bronquial en el sexo femenino.

El asma bronquial es muy frecuente entre los catorce y cuarenta años, o sea en las etapas de la vida en que el ser humano desarrolla su máxima capacidad física y reproductiva.

La existencia del asma de tipo atópico en un 45 por 100 de los casos estudiados.

La rinitis fue la manifestación alérgica previa más común en nuestros casos.

La mayor frecuencia de las crisis en la estación lluviosa y calurosa,

probablemente por la proliferación de las esporas de hongos saprofitos de la atmósfera.

Las asociaciones más frecuentes fueron la rinitis, la conjuntivitis y la dermatitis atópica.

Pudimos comprobar la existencia de un componente infeccioso en el 20 por 100 de los casos en estudio.

Entre las sensibilizaciones medicamentosas las más frecuentes fueron la penicilina, la aspirina, el yodo, la efedrina y la tetraciclina.

El estudio de las sensibilizaciones por inhalantes, dando prioridad a los hallazgos clínicos sobre los del laboratorio en la muestra estudiada, encontramos que los inhalantes más frecuentes fueron: polvo doméstico, hongos saprofitos de la atmósfera, emanaciones de insectos, sustancias de uso frecuente en el hogar: lana, lirio de florencia, plumas, algodón, kapok, papel periódico.

En último término colocamos a los pólenes, en nuestro medio la fiebre de heno y el asma estacional, que es una afección desconocida. Los investigadores que han realizado recuentos de pólenes atmosféricos han encontrado recuentos bajos, si se comparan con los de los países de la zona templada.

La sensibilización para alimentos de acuerdo a anamnesis y a las pruebas de eliminación y exposición, revela que los principales sensibilizantes en la edad adulta va en orden de frecuencia: los huevos, el pescado, la leche, el trigo, las caraotas o frijoles negros, las naranjas, el maíz y el banano.

THE INCIDENCE OF AETIOLOGICAL FACTORS IN BRONCHIAL ASTHMA IN THE NETHERLANDS

W. J. QUARLES VAN UFFORD.

Introducción.

The Dutch investigator will have difficulty in answering the question put as there is considerable confusion regarding the definition of asthma to-day. Is the symptom complex termed asthma a separate morbid entity after all or should it be classified with the large group of chronic non-specific respiratory diseases? The confusion becomes particularly apparent when the morbidity rates of asthma are considered. About 1950, the incidence was assumed to range from approximately 0.9 to 1.1 per cent, whereas to-day it is claimed to be as high as eight per cent.

The definition suggested by WILLIAMS (Cardiff) has been adopted in the present report wherever possible.

Mortality statistics have not shown any marked changes in recent years.

Central Bureau of Statistic, The Hague:

"Mortality rates since the introduction of the new code system used in (allergic) bronchial asthma:

	Men	Women	Total	Total number of deaths
1958	220	150	375	84,175
1959	209	135	344	85,752
1960	179	150	329	87,486
1961	158	115	273	87,923
1962	183	130	313	93,558

These are cases in which bronchial asthma was the primary cause of death.

Cases in which bronchial asthma was a contributory and therefore secondary cause of death:

	Men	Women	Total
1958	88	63	151
1959	78	61	139
1960	73	51	124
1961	104	138	187
1962	138	92	230

The increase during the period from 1961 to 1962 is due to the fact that the inclusion of secondary causes of death has widened the scope of coding."

Another problem is that of furnishing proof of a particular cause. Is this a matter of reproducing attacks or of collecting circumstantial evidence (such as stigmata indicative of an allergic constitution, the history of the patient and positive skin tests) ?

This also is a controversial point.

The majority of authors agree, however, that asthma is hardly ever due to a single cause without any other predisposing causes. On the other hand, authors are in agreement on the fact that there are multiple causes and multiple conditions.

The following data on precipitating causes was provided by the histories of patients: diseases and other factors preceding the onset of allergic manifestations.

	Bronchial asthma		Bronchial asthma
apparent cold	196	menarche	
bronchitis	171	military service	4
pityriasis rosea	104	scarlet fever	3
whooping cough	55	smallpox vaccination	2
spontaneous onset	49	climateric	
pneumonia	32	infectious hepatitis	2
rhinitis	20	having witnessed an attack of asthma in another individual	2
moving house	16	detention in a concentration camp	2
nervousness	16	otitis media	1
confinement	15	sciatica	2
influenza	15	malaria	1
measles	11	course of treatment with penicillin	1
being overworked	11	change of climate	1
occupational factors	10	change of dietary habits	1
course of treatment for tuberculosis	9	exposure to intense sunlight.	1
tonsillectomy	9	playing on grass, etc.	
surgical treatment	7	examination	
acute form following severe fright	6	cause obscure	205
pleurisy	5		
periodical school-instruction	5		
Vincent's angina	5		
diptheria	5	Total	1,000

ALLERGIC CAUSES.

Van der BIJL, Amsterdam, supplied the following data:

house-dust, approx.	5.79 %	
filling material for pillows and mattresses	2.55 %	
pollen	1.85 %	
fungi	2.78 %	
bacteria	15.50 %	(Haemoph., pneumococci, Neisseria, staph., Klebsiella, streptoc.)
food	2.86 %	

2.000 patients, 696 of whom showed some form of allergy.

H. J. ten Cate, Rotterdam:

The following is a rough estimate. Patients responding to combinations of allergens such as house-dust and food allergens have been included.

house-dust.	30 %	(Filling material for pillows and mattresses comes under house-dust in almost every case.)
pollen	15 %	
fungi	5-10 %	
bacteria	5-15 % ?	Probable allergy
food	30 %	

Asthma, rhinitis, atopic dermatitis and urticaria as well as migraine have been included.

These findings are so interesting because almost anyone knows that the proportion of positive skin tests with house-dust is much higher (about ninety per cent in cases of allergy).

When results of skin tests made in (allergic) asthmatic patients are compared with those obtained in cases of dermatitis, urticaria or colitis, reactions to inhalant allergens are found to be much more intensive than are those to food allergens. The very intensive reactions to gram-negative bacteria previously were a striking feature in cases of iridocyclitis and uveitis, whereas these were smaller and rarer in asthmatic patients.

The house-dust allergen undoubtedly is the most important allergen in the Netherlands. The question of whether it is a single polysaccharide or consists of several polysaccharides or whether it is a cocktail marked by hypersensitiveness to one of its constituents, has not yet been answered. A very preliminary study using house-dust extracts prepared during different months of the year indicated that apparently there were marked individual variations.

It is precisely pollen asthma which drives patients to see the allergist. While ORL-symptoms and fatigue are accepted as annoying seasonal manifestations, patients are seriously distressed by attacks of dyspnoea at night, which mainly occur during the second period of the hay-fever season (from late in May to the middle of July); patients seek rooms far from the open air (a cinema manager sat in his empty cinema hall at night in order to be relieved of his symptoms).

It would be interesting to know the extent to which pollen asthma occurs (directly, from one year to another) in untreated patients with hay fever, in those treated with antihistaminics or even in desensitized patients.

Testing for hypersensitiveness to fungi is a major problem, particularly as it is extremely difficult to prepare good extracts. Van der Werff is still engaged in intensive studies on the subject.

As regards filling material for pillows and mattresses, a differentiation should be made between fresh fillings (kapok, feathers, Alpine grass) and the contents of old mattresses and pillows (old kapok, etc.).

Genuine hypersensitiveness to animal hair is hardly ever observed by allergists. This probably is also due to the fact that these patients establish the diagnosis themselves and adopt their own measures.

Asthma due to food allergy is observed. Thanks to Kremer and De Lind van Wijngaarden, its possible presence continues to be taken into account and efforts are made to identify food allergens (food, flavour).

Infection.

There is no definite answer to the question of whether bronchitis is a cause, a predisposing cause, a complication or a sequela. Allergists are only too inclined to draw conclusions as to the cause from the results obtained in treatment. When treatment with antibiotics or an (autogenous) vaccine has been successful, however, this does not imply that the patient underwent causal therapy.

The following results were obtained on determination of the antistaphylolysin and antistreptolysin titres: In approximately 650 asthmatic patients:

Antistreptolysin titres were increased in about thirty per cent of the cases (over 250);

Antistaphylolysin titres were increased in about forty per cent of the cases (over 0.5);

Anticoagulase titres were increased in about forty per cent of the cases (Acta Medica Yugoslavica XII, 3, 1959).

There are four features which suggest that there may be an important relationship between infection and asthma:

a) Asthma in pre-school children, which frequently follows whooping cough, measles or bronchitis (cases of tracheobronchitis should not be included in this group);

b) The frequent coughing of asthmatic patients (studies by Nolomans with a recently constructed cough-meter in the TNO Department

of Physico-medical Research); with reference to this fact, it should be stressed that this coughing does not serve to eject mucus but that the sputum contains polynuclear leucocytes and bacteria;

c) The frequently increased erythrocyte sedimentation rate (approximately thirty per cent of the cases);

d) The seasonal peak in February in which month symptoms of bronchitis are common (this often results in hospitalization).

A passing reference should be made to the view held by a large number of general practitioners (which view is difficult to verify) that their asthmatic patients were tided over the winter by inoculation against influenza in some cases, by long-term antibiotic therapy (prevention) in others and by a simple course of treatment with artificial sunlight in yet others.

Endocrine factors.

Which are the effects of, for instance, the pituitary-adrenal system?

Low blood glucose tolerance curves, low blood glucose levels in fasting patients or low potassium levels of the serum are present in a number of cases. Determination of the urinary elimination of 17-ketosteroids and 17-hydroxycorticosteroids in a large number of cases lent little support to the theory that diminished function of the adrenals is a common feature of an allergic constitution.

The number of cases studied is still too small but the results obtained in determinations made prior to, during and shortly after pregnancy may possibly explain why attacks of asthma usually disappear during pregnancy and recur or start immediately after pregnancy.

It continues to be obscure which endocrine factors are of importance in so-called postclimateric asthma. The results obtained using lyndiol in the treatment of the syndrome of premenstrual asthma are reported as being encouraging (inhibition of ovulation).

Mental factors.

Genuine nervous asthma is rare. The studies by Stokvis (the leading worker in the field of psychosomatic research in the Netherlands, who unfortunately died in 1963) are of the utmost importance to those who believe themselves to be justified in concluding that asthma is the result of mental conflicts when the latter are associated with asthma. Stokvis showed that chronic asthma has an effect on the mind. One of his coworkers published an excellent report on a case in which asthma and mental symptoms alternated with each other.

It is only too apparent that mental factors predispose to asthma: aggravation of asthma in children at or immediately after St. Nicholas, dropping out of members of the staffs of shops on particularly busy days.

The following are three important predisposing causes which were identified during detailed social studies in a large number of asthmatic patients;

a) Wanting or having to aim too high mentally, standing on tiptoe mentally, either when working or in social intercourse.

b) The fact that it is extremely difficult to exert a therapeutical effect on patients belonging to a particular religion. Certain groups of Protestants have such a sense of sin, or being excluded from all grace, that any joy of life is banned. It is difficult to exert any effect on patients of this cheerless group.

c) Birth rate (position in the family) and the onset of asthma. Studies on the subject are particulary difficult. At best, it can be stated that a partial loss of interest on the part of the mother may act as a predisposing cause.

The following table was published previously as the result of a social-medical study (in 100 asthmatic patients).

Findings in social studies in 100 cases of bronchial asthma.

Childhood spent in disharmonious environment	24 %	(Parents at odds, father given to drink, immorality.)
Oversolicitous mother (attachment to the mother)	15 %	
School	2 %	
Home (overcrowded, dusty, fungi)	35 %	
(Potent, unhealthy sources of allergens —factories, etc.— in the surrounding area.)	3 %	
Occupation	5 %	(Serious trouble with employers, foremen, etc.)
Certified 100 % disabled	5 %	(One of these very satisfied as he can now have a small shop of his own.) (The patient —not named was several times successful in prolonging the period of his disease to the longest possible extent, though just so long that he could avoid being examined for disability.)
Religion (very religious)	30 %	
(consciously irreligious)	8 %	
Spinsters and bachelors: disharmonious environment	5 %	
Married persons: many difficulties with husband or wife	7 %	(Unsatisfactory relationships in spite of genuine love.)
Excessive attachment to their children.	3 %	(The difficulties started when children married or when a favourite child left the home.)
Family problems, infidelity (and sense of guilt)	2 %	
Living above their mental station	5 %	
Anxiety	9 %	
Home satisfactory, environment satisfactory	20 %	(Neither the welfare worker nor the psychiatrist were able to detect any aberrations.)
Home unsatisfactory, environment satisfactory	7 %	

EEG and allergic disease

		Normal	Epilepsy	Epilepsy and migraine	Migraine
Bronchial asthma	299	191	69	22	17
Bronchial asthma and dermatitis	5	2	1	—	2
Bronchial asthma and migraine	5	2	1	1	1
Bronchial asthma and urticaria	1	1	—	—	—
	310	196	71	23	20

Social factors.

Dr. Syrier (engaged in an inquiry on behalf of the foundation "Study of the Social Consequences of Bronchial Asthma", which includes an investigation into social causes) obtained the following results as regards homes:

Moved house on account of their asthma	48
Subsequent improvement in state of health	33
No subsequent improvement in state of health	13
Cannot be assessed	2
Adequate home	109
Inadequate home	41 or 20.7 %

Occupational factors.

Men

No change of occupation, continued in same occupation	10
No change of occupation, occupation discontinued on account of asthma	7
Changed occupation once on account of asthma	10
Changed occupation once for other reasons	13
Changed occupation twice on account of asthma	6
Changed occupation twice for other reasons	4
Changed occupation three times on account of asthma	4
Changed occupation three times for other reasons	0
Changed occupation more than three times on account of asthma	1
Changed occupation more than three times for other reasons	1

Women

No change of occupation (discontinued on account of marriage in the majority of cases)	25
No change of occupation, discontinued on account of asthma	7
Changed occupation once on account of asthma	2
Changed occupation once for other reasons	12
Changed occupation twice on account of asthma	4
Changed occupation twice for other reasons	1

As regards occupational causes, it can be stated that employment with industries in which work is continuous was found to be an adverse factor.

Genuine occupational asthma is rare, though it may occur in bakers, hairdressers, nurserymen, etc.

Muller (Apeldoorn) observed several cases at a codeine factory.

The extent to which an allergic constitution may act as a predisposing cause in workers employed in mines and in the textile industry continues to be open to question.

Season, Climate, Weather, etc.

From the middle of August until the first night-frost appears, there is an increase in the number of cases hospitalized for status asthmaticus and allergists are consulted by an increasing number of patients for aggravation or even for recurrence of their symptoms after these have been in abeyance for many years. Three factors might be suggested continues to be open to question.

a) House-dust allergy (altered habits of life, more living indoors).
b) Fungus allergy a sa cause or predisposing cause.
c) Humidity as a predisposing cause.

District and Town.

Storm von Leeuwen already found the incidence of symptoms of asthma to differ in various parts of the Netherlands. This was verified by a map of the incidence of asthma, which was drawn for the first IAA Congress.

"Large towns" and "humid districts" are the main factors in this case. Removal to a dry district free from air pollution may occasionally produce some improvement. Air-pollution apparently also acts as a predisposing cause in a number of municipalities along the New Waterway (Rotterdam-Hook of Holland), where there are many large industries. As early as several years ago, Belonje reported an aggravation of asthma in children at the seaside, which aggravation only occurred during July and August.

Habits of life.

In view of the Dutch climate, much swimming has to be done in covered swimming-baths (chlorine vapours). Muller (Apeldoorn) pointed out that when patients cease frequenting swimming-baths, this will frequently result in the disappearance of a chronic unproductive cough in asthmatic patients.

Smoking continues to involve a hazard of emphysema in asthmatic patients, though it seldom causes an attack of asthma.

As a result of the geographical position of the Netherlands (many harbours), a large number of workers are employed in the docks (specific and non-specific contacts with goods imported from all parts of the world).

SUMMARY.

"The Netherlands" are the country of water (humidity) and pastures (grass pollen), of honey (beware of pollen allergy) and cheese (fungi in cheese-warehouses), flowers and fruit (occasional cases of asthma in greenhouses (tomato-farms) and orchards (due to the non-specific stimulus of chemical spraying agents).

It is the country of farmers (outbreaks of asthma when cattle are stabled), of industry (much new construction and therefore adequate drainage systems) and of overpopulation (dust, etc.), tourist traffic and relative prosperity (much exhaust gas from motor-cars).

Perhaps the very fact that it is a small and densely inhabited country is the reason why the possibility of multiple predisposing factors (humidity, air-pollution, infection) as well as national characteristics (psychogenic factors) and the role of several causes: large numbers of allergens (dust, fungi, pollen, diet, bacteria) have to be stressed to this degree.

Unfortunately, this increases the difficulty in presenting a review of the incidence of aetiological factors in bronchial asthma.

FACTORES ALERGOGENOS CONDICIONANTES DEL ASMA BRONQUIAL EN COLOMBIA S. A.

M. SÁNCHEZ MEDINA (*), C. DE STRIEGEL (**)

Se limita esta comunicación a presentar los estudios iniciales aerobiológicos (micológicos y polinológicos), la revisión numérica de los casos de asma en los principales hospitales de Colombia y el análisis de otros posibles factores alérgicos condicionales del asma en el medio nacional.

Hongos alergógenos.

En el medio aéreo se presenta el resultado de la exposición semanal durante un año, que establece los géneros de hongos en orden de preponderancia y la posible relación con las características climáticas de cada lugar.

En cada una de las ciudades que más adelante se discriminan, se hizo la exposición cada siete días, abriendo una caja de Petri durante quince minutos en el sitio elegido, puesta a temperatura ambiente por un lapso de cuatro a ocho días y advirtiéndose que en las zonas cálidas se realizó en tiempo menor (diez minutos) que en las zonas frías.

Se determinó el orden, la frecuencia e incidencia de los géneros de hongos clasificados microbiológicamente.

El resultado de la lectura de las exposiciones que recolectan los hongos se presenta separando cardinalmente las regiones de Colombia y se tiene en cuenta asimismo la situación geográfica de cada ciudad o lugar (altiplanicie, montaña o llano), el clima (cálido, medio o frío), la humedad del ambiente, el régimen de lluvias y el índice de población.

Las exposiciones se hicieron en las siguientes regiones de Colombia; junto con ellas se numera el orden de frecuencia anual de los géneros que se identificaron; estos mismos resultados se presentan en cuadros mensuales separados para cada región.

1. *Sabana de Bogotá* (Bogotá, Chía, Zipaquirá, Ubaté y La Unión).

El orden de frecuencias de las colonias halladas fue: Penicillium,

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Aspergillus, Fusarium, Hormodendrum, Mucor, Alternaria, Streptomyces y Cephalosporium.

En cantidad menor a 2 colonias mensuales se hallaron: Rhizopus, Scopulariopsis, Rhodotorula, Trichotecium y Helminthosporium.

2. *Región de la Cordillera Central.*

a) *Manizales.*—Penicillium, Hormodendrum, Mucor, Fusarium, Alternaria, Phoma, Cephalosporium, Rhizopus, Verticilium y Candida. En cantidad menor a dos colonias se hallaron: Paecilomyces, Streptomyces, Cephalosporium, Aspergillus, Rhodotorula.

b) *Pereira.* — Penicillium, Hormodendrum, Fusarium y Phoma; menor a dos colonias se hallaron : Alternaria, Cephalosporium, Rhodotorula y Pullularia.

c) *Armenia.* — Hormodendrum, Aspergillus, Phoma y Candida. Para cajas con menos de una colonia semanal, igual a lo hallado en Pereira.

d) *Medellín.* — Penicillium, Fusarium, Aspergillus, Hormodendrum; en cantidad menor a dos colonias se identificaron: Pullularia, Trichoderma, Trichothecium, Stemphillium, Rhizopus, Mucor, Alternaria, Streptomyces y Rhodotorula.

3. *Región Costa Atlántica.*

a) *Barranquilla.* — Hormodendrum, Aspergillus niger, Fusarium, Stemphillium, Trichothecium, Penicillium, Aspergillus, Monilia.

En cantidad menor a dos colonias se hallaron: Rhodotorula, Streptomyces, Helmintosporium.

b) *Cartagena.*—Penicillium, Aspergillus niger, Fusarium y Tricothecium.

Los demás hallazgos fueron similares a los de Barranquilla para número menor a dos colonias.

c) *Santa Marta.*—Aspergillus niger, Aspergillus fumigatus, Penicillium, Hormodendrum, Aspergillus orizae, Scopulariopsis, Fusarium.

Menor a dos colonias: Cephalosporium, Levaduras, Monilia sitoifila, Pullularia y Mucor. En menor número se hallaron los mismos hongos identificados en Barranquilla.

d) *Sincelejo.* — Hormodendrum, Penicillium, Alternaria, Candida, Trichoderma. Se identificaron además en colonias muy escasas los hongos hallados en Barranquilla.

4. *Región del Valle del Cauca.*

a) *Cali.*—Penicillium, Diplosporium, Fusarium, Rhizopus, Hormodendrum, Aspergillus, Mucor. Menor a dos colonias semanales, Phoma, Cephalosporium y Trichotecium.

b) *Cartago.*—Penicillium, Rhizopus, Fusarium, Hormodendrum, Pullularia, Aspergillus. Menos de dos colonias, Trichotecium, Phoma y Cephalosporium.

5. *Región Costa Pacífica Sur.*

Tumaco.—Penicillium, Aspergillus fumigatus, Aspergillus orizae, Verticillium, Streptomyces, Fusarium, Monilia sitofila, Helmintosporium, Scopulariopsis, Cephalosporium y Botrytis.

6. *Región del Alto Magdalena.*

a) *Barrancabermeja.*—Fusarium, Hormodendrum, Aspergillus niger, Aspergillus Sp., Alternaria, Penicillium.

b) *Honda.*—Aspergillus Sp., Fusarium, Streptomyces, Cephalosporium y Levaduras.

c) *Girardot.*—Aspergillus niger, Hormodendrum, Penicillium, Candida, Fusarium, Candida albicans y Aspergillus fumigator.

d) *Neiva.*—Hormodendrum, Aspergillus niger, Fusarium, Phoma y Pullularia.

7. *Región Nor. Oriental (Santanderes).*

a) *Cucuta.*—Aspergillus orizae, Penicillium, Hemispora, Cephalosporium, Levaduras, Monilia Sp., Aspergillus Sp., Hormodendrum.

b) *Bucaramanga.* — Fusarium, Hormodendrum, Mucor, Rhizopus, Pullularia; menos de dos colonias semanales, Alternaria, Trichotecium, Aspergillus niger, Aspergillus flavus, Trichoderma, Phoma, Trichotecium y Levaduras.

8. *Región Llanos Orientales.*

a) *Villavicencio.*—Hormodendrum, Fusarium, Penicillium, Cephalosporium.

b) *Orocue.*—Mucor, Aspergillus niger, Penicillium, Pullularia, Diplosporium, Hormodendrum y Rhodotorula.

9. *Región Central de Boyacá.*

Sogamoso.—Penicillium, Aspergillus, Pullularia, Fusarium, Hormodendrum. Para cajas con menos de dos colonias semanales los resultados son muy semejantes a los hallados en la Sabana de Bogotá.

Pólenes alergógenos.

Solamente fue posible determinar el calendario polínico en tres zonas de la nación: a) Costa Atlántica (Barranquilla), b) Sabana de Bogotá (Bogotá D. E.) y c) Valle del Cauca (Cali), para donde se tomaron las referencias de Naranjo [1].

Para la Sabana de Bogotá [2] se establece que las familias más frecuentes y posiblemente de mayor responsabilidad alergógenas son:

1) Las gramíneas, cuya época de polinación definida se encuentra durante los meses de noviembre, diciembre y parte de enero.

2) Ocupan el segundo lugar otras plantas herbáceas y algunas arbustivas, generalmente mal llamadas "malezas", cuyos períodos de polinación son semejantes a los que tienen las gramíneas, pero con un intermedio en los meses de junio y agosto.

3) En tercer lugar están los árboles, de los cuales el de mayor frecuencia en cantidad de polen es la Acacia Sp., dominante en la ornamentación de Bogotá, cuya polinación está claramente determinada para los meses de junio, julio y parte de agosto, con una nueva florescencia en diciembre.

En la Costa Atlántica (Barranquilla) las gramíneas tienen su época de polinación en diciembre y parte de noviembre. De abril a junio aparecen las "malezas" (amarantáceas y quenopodiáceas), cuyo número de granos de polen, en las láminas expuestas, es menor que el de las

gramíneas. Los árboles tienen su mayor polinación en los meses de febrero y marzo.

Para el Valle del Cauca, según Naranjo [3], "el número de pólenes por centímetro cuadrado, en láminas y en veinticuatro horas, sólo excepcionalmente llega a diez o más". "Hay ciertas diferencias en cuanto a las especies botánicas predominantes; en la atmósfera de Cali se encuentran más pólenes de árboles (leguminosas, bigonáceas)." "En cuanto a las amarantáceas, Naranjo menciona en Cali al polen de Amarantus spinosus; hay también apreciable número de pólenes de gramíneas."

"El asma y la alergia vasomotora existen en las zonas estudiadas." "Su frecuencia relativa varía de un lugar a otro." "En Cali las alergias respiratorias, particularmente el asma, son más frecuentes que las alergias cutáneas." "La altitud no parece influir, ya que sobre pacientes hospitalizados se encuentra aproximadamente la misma frecuencia de asma en Bogotá, que está a 2.630 metros sobre el nivel del mar, que Cali, que está a 1.000 metros."

Alimentos alergógenos.

En Colombia, la alergia alimentaria no puede ser valorada con certeza como agente etiopatogénico específico del asma, ya que el consumo alimentario varía según las condiciones económicas y las regiones del país. Además no hay estudios concretos hechos por alergólogos especializados en ciudades diferentes a la de Bogotá, en cuanto a alimentos causantes con mayor frecuencia del asma alérgica.

Cuervo Trujillo [4] considera al orden de frecuencia de alergia alimentaria así: "a) Frutas (naranja, piña, aguacate, tomate de árbol o de monte, coco, mango, melón, papaya, fresas); b) Cereales: arroz, maíz, trigo, avena; c) De origen animal: carne de cerdo, carne de res, leche, huevos, peces, mariscos, crustáceos; d) Leguminosas: frijoles, habas, soya, arvejas, maní, lentejas; e) Verduras y legumbres: espinacas, coles, coliflor, repollitas, apio, tomate, zanahoria, papa, plátanos; f) Bebidas: café, chocolate; g) Condimentos: esencias, resinas, gomas, mostaza, ají, cebolla, ajo, pimienta, canela, vainilla, clavos, especias."

En el asma alérgico consideramos que puede haber cierta relación con la frecuencia del consumo de determinados alimentos, que según los datos que arrojan las estadísticas de Colombia [5] es el siguiente: café, plátano, arroz, tubérculos (papa, yuca y arracacha), leguminosas (frijol, garbanzos, arvejas y lentejas), carne, leche, huevos y otras frutas.

Se supone en consecuencia que para la población de mejores posibilidades económicas, la variedad y calidad de la ingesta alimentaria varíe notablemente, ya que los alimentos de mayor contenido proteico tienen mayores posibilidades de ser adquiridos y, consecuentemente, determinar mayor número de casos de alergia por alimentos.

Falta por determinar la capacidad alergógena de cada alimento para las distintas zonas de Colombia, labor ardua a realizar en un medio de tan pocas posibilidades de diagnóstico en alergia como el nuestro.

Asma alérgica por infección y bacteriana.

VILLAVECES y DE ZUBIRÍA [6] consideran que las asmas producidas por infección (focos orgánicos de habitual localización bacteriana) pueden tener la misma incidencia general que la relatada por alergólogos de otros países. Son las ciudades en donde puede presentarse mayormente el asma de esta patogenia, en razón de la densidad de la población si se compara con los lugares menos poblados, en donde la consulta por asma es menos frecuente.

No parece probable que los factores endógenos, bacterianos y/o parasitarios varíen significativamente en comparación con las referencias de la literatura universal sobre este tópico. Sin embargo, DE STRIGEL [7] considera que por estudios microbiológicos realizados en 500 pacientes con signos de alergia en el tracto respiratorio, existen agentes microbianos determinados, tales como estreptococo beta-hemolítico, estafilococo aureus homolítico coagulasa positiva y neumococo, los cuales se muestran como responsables del posible componente alérgico bacteriano. Esto lo demuestra este autor por los títulos elevados de antiestreptolisinas y la respuesta clínica única a la antibioterapia de elección.

Incidencia del asma en los hospitales de Colombia.

Con VILLAVECES [7] realizamos una encuesta en todos los hospitales de Colombia vinculados directa o indirectamente con el Ministerio de Salud Pública, en un total de 567. En cuadros separados se trascriben los resultados de tal investigación, la cual permite apreciar en forma apenas preliminar la posible incidencia de la enfermedad asmática en la nación. La cifra global de frecuencia de asma en los hospitales encuestados da un promedio anual de 5,4 por 100.

NARANJO [8] considera la frecuencia relativa de asmáticos hospitalizados sobre un promedio de enfermos/año en Bogotá de 6,3 por 100, para 11.000 enfermos. En Cali, 6,7 por 100 para 5.500 enfermos.

RESUMEN Y CONCLUSIONES.

1.° Se presentan los estudios iniciales aerobiológicos de la frecuencia de géneros de hongos en determinadas regiones de Colombia: Sabana de Bogotá, cuatro ciudades de la cordillera central, Costa Atlántica, Valle del Cauca, Costa Pacífica Sur, Alto Magdalena, región nor-oriental (Santanderes), Llanos Orientales y región central de Boyacá. Los géneros de hongos encontrados en las principales regiones de Colombia, según su orden de frecuencia, son: Penicillium, 53,39 %; Hormodendrum, 22,47 %; Fusarium, 6 %; Pullularia, 4 %, y Aspergillus, 3,8 %; los otros géneros: Rhizopus, Streptomyces, Levaduras, Alternaria y Mucor, presentan frecuencia muy variable para las distintas regiones estudiadas.

2.° Se presenta el calendario polínico de tres regiones cardinales de Colombia; altiplanicie de la Sabana de Bogotá, Costa Atlántica (Barranquilla), y se transcriben los resultados obtenidos por NARANJO para el Valle del Cauca (Cali).

3.° Se analizan otros factores alérgico-condicionantes en el medio nacional: alimentarios, bacterianos y parasitarios.

4.° Se presenta el resumen de la encuesta efectuada en 567 hospitales de Colombia sobre la incidencia de asma (5,4 por 100) en los enfermos que aquéllos consultaron.

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FRÉQUENCE ÉTIOLOGIQUE DE L'ASTHME BRONCHIQUE EN ITALIE

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L'A. au moyen exclusif de diagrammes figuratifs, décrit l'Italie comme une péninsule de 301.021 km. carrés, peuplée d'environ 52.000.000 d'habitant, reliée au Nord à l'Europe centrale et dont la caractéristique forme de botte s'avance, avec 8.750 km. de côtes, dans la Méditerranée jusqu'à pouvoir apercevoir les côtes de l'Afrique Septentrionale d'une distance d'environ 140 km. Cette situation géographique détermine quatre types de climats qui conditionnent la flore, la faune, les mœurs et coutûmes de ses habitants, le développement industriel, le commerce, les moyens de transport, la pureté et les contaminations atmosphériques. Ces quatre climats sont les suivants: le climat Alpin, celui de la Plaine du Pô, le climat Méditerranéen et enfin le climat péninsulaire.

En se basant sur ses propres statistiques et sur celles de savants qui seront nommés au cours de la projection, l'A. croit pouvoir fixer à 2.400.000 le nombre des bronchiteux chroniques en Italie et à 1.600.000 celui des allergiques. Parmi ces derniers l'asthme bronchique est représenté dans la mesure du 75 %.

Les causes etiologiques de ce 75 % c'est à dire d'environ 1.200.000 asthmatiques peuvent être ainsi subdivisées: allergie contrôlée du 50 au 60 %, allergie bactérique du 25 au 30 %, allergie par cause indirecte du 1 au 3 % cause psychique du 1 au 2 %, par causes mixtes du 2 au 17 %; causes indéterminées ou non déterminables du 0 au 21 %.

Les asthmatiques adultes forment le 72-94 %, la différence est représentée par les enfants au-dessous de 12 ans. L'asthme est généralement légèrement plus répandu chez les hommes que chez les femmes.

La région qui compte le plus petit nombre d'asthmatiques est la Vallée d'Aoste, celle qui en compte le plus grand nombre est la Lombardie, suivie de la Campanie e du Piémont. Les autres 18 régions se trouvent dans l'arc compris entre le Piémont et la Vallée d'Aoste.

Sur 100 cas d'asthme bronchiale allergique le 50 % est représenté par des allergiques respiratoires; le 20 % d'allergiques alimentaires; le restent 10 % divisé en parties égales entre allergiques aux produits pharmaceutiques, bactériques et endogènes.

Parmi les allergiques respiratoires le 50 % est représenté par la poussière de la maison, par les moutons qui se forment sous les meubles

et par les pollens; le 10 % par les poussières industrielles; le 10 % par la desquamation d'animaux domestiques; le 10 % par les fibres textiles naturelles (coton, lin, chanvre); le 10 % par la farine de froment; le 5% par les fibres textiles artificielles (nylon, rayon, lylion, etc.); le 3 % par les fibres textiles animales (laine et soie); le 2 % par les poussières végétales.

Les allergènes alimentaires sont ainsi distribués: 28 % oeufs; 17 % viande de porc; 10 % lait entier; 10 % mollusques et fruits de mer; 9 % farines de céréales (presque exclusivement froment); 8 % laitages; 8 % légumes et fruits; 6 % viande de boeuf; 2 % chair de poulet; 2 % aliments variés.

Les raisons climatiques dont nous avons parlé plus haut et qui conditionnent aussi les moeurs et coutumes, les cultures etc. font que la distribution des allergènes alimentaires varie de région à région. En Sicile et en Sardaigne on trouve des allergies aux fèves, allergies qui sont inconnues dans les autres provinces d'Italie. Les pollens de palmier et d'autres plantes sub-tropicales sont notables seulement en Ligurie et en Italie Méridionale. Les pollens des urticacées sont fréquemment allergènes dans la partie centrale et méridionale de la péninsule, tandis qu'ils le sont beaucoup moins en Italie septentrionale.

Parmi les pharmacoallergènes la majorité est représentée par les antibiotiques, par l'aspirine, par les salicilates, par les sulfamides, par les barbituriques. Les produits organothérapiques causent fréquemment l'asthme bronchique.

Allergènes variés: les rayons du soleil, le smog, fumée de charbon végétal ou mineral. le mazout, le pétrole, le soufre.

L'asthme professionnel est catalogué en ordre décroissant: travailleurs affectés aux transports lourds, ouvriers affectés à des produits chimiques, souffleurs de verre, ouvriers tisseurs du coton, ouvriers affectés aux entreprises d'extractions, ouvriers dans l'ndustrie textile sinthétique, dans les exploitations agricoles, industries alimentaires, maçons et ouvriers dans la fabrication du ciment, céramistes, ouvriers dans l'industrie lainière, ouvriers dans les fabriques de papier, dans l'industrie de la soie, menuisiers, cordonniers.

En 1954 sur un groupe de 5.865.553 individus assistés par l'Institut National d'Assistance contre les Maladies, on a noté 30.761 cas d'allergie dont un tiers d'allergie asthmatique, causant une perte de 491.509 journées de travail. Pensons que la population italienne s'élève à 52.000.000.

Les Professeurs SERAFINI, CAPUANI, GIRO et les docteurs ALICE, PIARDI et PATERA, appartenants à des differentes régions d'Italie, ont collaboré en fournissant leurs observations, à la rédaction de la présente relation et l'Auteur les en remercie vivement.

FREQUENCE DES FACTEURS ETIOLOGIQUES DE L'ASTHME BRONCHIQUE EN YOUGOSLAVIE

V. SPOUJITCH.

Il y a une vingtaine d'années que nous résolvons ce problème complexe en Yougoslavie. Etant donné que tous les habitants ne sont pas membres de la Sécurité sociale et que les stations sanitaires, surtout dans les villages reculés, ne sont pas dirigées par des spécialistes-allérgologues, il ne nous a pas été possible d'utiliser les données fournies par ces institutions. Cependant, afin de pouvoir répondre à la question qui se posait, nous avons formé une équipe d'une quinzaine de spécialistes expérimentés: interniste, allergologue, dermatologue, pédiatre, oto-rhino-laryngologue, cardiologue, neurologue, higiéniste biochimiste, radiologue et d'autres, dont la mission était d'examiner l'état de l'allergie des habitants des régions les plus diverses de Yougoslavie. Les habitants de certaines plus grandes villes on été examinés, aussi bien que ceux des hameaux reculés dans des régions montagneuses difficilement praticables, puis les ouvriers de différentes industries (des textiles, de la farine, du tabac, du caoutchouc, de la métallurgie, etc.) et les agriculteurs des régions où l'on fait la culture de certaines plantes industrielles (ricin, chanvre, riz, paprika, etc.). En même temps, une attention particulière a été portée aux conditions géoclimatiques des régions en question, au type des agglomérations, à l'état économique, au mode de vie ainsi qu'aux conditions d'hygiène du travail des travailleurs.

On pourra peut-être reproché à notre méthode d'examen qu'elle n'est pas appliquée à toute la population, ce qui est encore aujourd'hui irréalisable, et que le diagnostic des manifestations allergiques est souvent posé d'après le premier examen. Pourtant cette méthode présent de nombreux avantages en comparaison aux autres, si cellesci ne disposent pas des données nécessaires exactes. Avant tout, au point de vue du diagnostic, car c'est toujours la même critérium qui est appliqué dans toutes ces conditions si diverses. L'exactitude du diagnostic, lors de ces examens sur le terrain, était garantie d'abord par le fait que l'attention de toute l'équipe était concentrée sur ces quelques cas d'asthme et d'autres manifestations allergiques. Ces cas étaient examinés dans le concilium de tous les spécialistes intéressés et, si besoin était, ces malades étaient souvent à nouveau examinés ou bien entraient dans certaines cliniques pour y être soumis à des examens complémentaires. De plus, en choisissant pour ces recherches des localités et un milieu où l'influence de certaines conditions se manifestait particulièrement,

nos examens ont souvent presque correspondu à de véritables expériences; enfin, en procédant à l'examen comparé de l'apparition des manifestations allergiques avec les facteurs locaux du milieu dans lequel ces manifestations étaient apparues, le rôle des conditions en question s'imposait de lui-même.

En 20 ans l'équipe a examiné environ 50.00 personnes des régions les plus diverses de Yougoslavie. Pendant chaque examen une moyenne de 300 à 1.000 enfants, femmes et hommes de différents professions ont été examinés.

Etant donné qu'en Yougoslavie, sur une étendue relativement peu grande, nous rencontrons des facteurs géoclimatiques et économiques très variés ainsi que des conditions de travail, de vie, des facteurs héréditaires, etc., très différents, nous avons pu y constater presque tous les facteurs qui jouent un rôle important dans le processe d'apparition de l'asthme et des manifestations allergiques. Ces facteurs si différents agissent pour la plupart conjointement.

Nous exposerons ici quelques facteurs qui ont le rôle le plus important dans l'étiologie de l'asthme en Yougoslavie.

La nature et l'abondance des allergènes sont d'une grande importance.

Chez les agriculteurs du Banat qui font la culture du ricin, nous avons constaté un pourcentage élevé d'asthme (2 %), de manifestations allergiques (6 %), de réactions cutanées positives à l'extrait de graines de ricin (22 %) et en général aux pneumallergènes (31 %). Les réactions locales aux tests cutanés avec l'allergène du ricin ont été fortes, et souvent suivies de manifestations allergiques: asthme, urticaire, etc. Nous avons pu faire une constatation analogue avec l'extrait d'Ascaris lumbricoides, chez les habitants des régions infestées par ce parasite. Ici aussi les tests cutanées avec l'extrait d'Ascaris ont provoqué souvent une réaction intense, accompagnée de manifestations allergiques: urticaire généralisé, crises d'asthme, choc anaphylactique faible ou plus grave. Contrairement à ces allergènes le pouvoir allergogène des autres allergénes est très faible. En Macédoine, chez les habitants qui travaillent dans les rizières, le porcentage de réactions cutanées positives au riz est insignifiant (1 %). Parmi eux il n'y a eu aucun cas d'asthme et de manifestations allergiques provoqués par le riz. De même il y a eu peu de tests cutanées positifs au chanvre, chez les ouvriers de l'industrie chanvrière du Banat et de la Serbie méridionale; nous n'avons constaté aucun cas d'asthme provoqué par le chanvre.

Les examens des ouvriers des différents ateliers de certaines fabriques (de textile, de soie, etc.) ont montré une augmentation nette du pourcentage de sensibilisés aux allergènes en question et de manifestations allergiques, dans les ateliers où ces allergènes étaient en abondance.

Les facteurs géoclimatiques sur le rôle important desquels STORM VAN LEEUWEN, FRUGONI et d'autres ont déjà attiré l'attention, se sont montrés en Yougoslavie également comme le facteur le plus puissant.

Dans notre pays aussi, au point de vue de l'asthme les régions les plus défavorables sont les régions de plaines, basses, inondables, situées le long des grandes rivières (Srem, Banat, Pomoravlje, etc.) où le terrain est inondable, le sol fertile, les habitations humides, l'air riche en allergènes de la poussière, des moisissures, des insectes qui attaquent le blé (DÍAZ) et autres. Dans les régions basses de Yougoslavie le pourcentage d'asthme atteint de 1 à 2 % et 3 % dans les plus grands centres industriels; celui des manifestations allergiques s'élève jusqu'à 6 %, celui des sensibilisés allant de 35 à 50 %. Au contraire, dans les régions montagneuses de Serbie, de Bosnie, de Macedoine, le pourcentage d'asthme est minimum et inférieur à 0'05%, celui des manifestations allergiques de 0,43 % et des sensibilisés de 6 à 18 %. A Lazaropolje (1.000 m. d'altitude), en Macedoine, nous n'avons trouvé aucun cas d'asthme même dans une fabrique de tapis.

L'hérédité, dont le rôle a déjà été mis en évidence par COOKE, HANHART, BRAY et d'autres auteurs a été soulignée également par nos recherches en Yougoslavie comme l'un des plus puissants facteurs. Dans les régions basses, asthmogènes (marécages de Pancevo), chez les habitants originaires des régions montagneuses et qui s'y sont fixés, y vivant depuis 10 et 15 ans, nous n'avons constaté aucun cas d'asthme. Chez les habitants de Lazaropolje (Macédoine) à 1.000 m. d'altitude, qui ont vecu et travaillé pendant 20 et 30 années dans des régions basses et asthmogènes pour y gagner leur vie (VOJVODINA, ROUMANIE et autres), nous n'avons pas non plus rencontré de cas d'asthme.

Pourtant, le développement particulièrement brusque de l'industrie dans notre pays, et qui n'est pas toujours accompagné des conditions nécessaires d'hygiène du travail, amène de plus en plus fréquemment l'apparition de l'asthme et des manifestations allergiques même chez ceux dont les ancêtres n'avaient eu ni asthme ni manifestations allergiques. L'asthme acquis devient chez nous aussi de plus en plus fréquent.

Presque tous les auteurs sont d'accord (HAIBE, SALÉN, DÍAZ, RACKEMANN et autres) sur l'importance du rôle de *l'infection* dans l'apparition de l'asthme chez les enfants et les adultes. Il n'y avait que différentes interprétations de l'action de l'infection, c'est-à-dire des bactéries. Avec DANILOVIC nous avons montré que les bactéries jouent parfois le rôle d'un allergène, mais que beaucoup plus souvent elles agissent dans la fixation des anticorps au tissu de choc, ce qui a été confirmé par PASTEUR VALLERY-RADOT, HALPERN et leurs collaborateurs. Nous avons constaté que dans la filature de soie à Novi Sad il y avait beaucoup d'asthmatiques dans l'atelier où les conditions de travail étaient la cause de fréquentes bronchites chez les ouvriers et que les crises d'asthme cessaient si les malades passaient dans un atelier où ces conditions, qui avaient amené la bronchite n'existaient pas. Dans la fabrique de lainage à Paracin où il y avait un pourcentage élevé de sensibilisés, mais où les conditions de travail n'avaient pas amené de bronchites,

il n'y a pas eu beaucoup de cas d'asthme. Chez les habitants de l'humide plaine pannonienne où, comme Haibe l'a trouvé en Belgique, les infections des voies respiratoires sont fréquentes, l'asthme l'est également, tandis que dans les régions montagneuses plus sèches et malgré des hivers plus rigoureux, les gens y sont plus résistants et l'on y constate aussi moins d'asthme. Sur le littoral dalmate de l'Adriatique, l'asthme apparaît le plus souvent à la suite de refroidissements, après des bains pendant les jours plus froids.

Le métier, la profession sont aussi un facteur très important dans l'apparition de l'asthme. Bien connus sont les asthmes des fourreurs, des coiffeurs, des meuniers, des pharmaciens et d'autres professions. Les examens comparés de l'état de l'allergie chez les ouvriers employés dans différents ateliers d'une fabrique et chez les habitants demeurant à proximité des fabriques ou loin de celles-ci (industrie sidérurgique en Bosnie), soulignent nettement le rôle de certains facteurs défavorables. Ce sont le plus souvent la viciation de l'air par la poussière ou des gaz toxiques ou l'humidité, les grands courants d'air, les grandes variations de temperature, etc. Il s'est avéré dans ces cas, comme dans des expériences, que le pourcentage d'asthmatiques était le plus élevé dans les ateliers où ces conditions défavorables étaient le plus fortement manifestées, qu'il était plus faible dans les autres ateliers de la même fabrique, encore plus faible chez les habitants demeurant à proximité de la fabrique, et le plus faible chez ceux qui habitaient loin de la source du facteur asthmogène en question. Nous avons mentionné que dans la filature de soie à Novi Sad, il était ordinairement suffisant de quitter l'atelier aux conditions de travail défavorables pour que les crises d'asthme cessent.

Le type des agglomérations exerce également une grande influence. Les plus défavorables sont les grandes villes surpeuplées, surtout leurs quartiers vieux et industriels aux rues étroites et sombres entre de hauts bâtiments de plusieurs étages. La proximité des marchés, des gares de chemins de fer, des fabriques, etc., agit aussi défavorablement. En Macédoine où il existe encore des villes du type oriental turc, aux rues étroites et sombres, aux maisons qui, avec leurs vérandas aux étages supérieurs, surplombent ces rues déjà obscures, au pavage turc, où les conditions de l'urbanisme et souvent aussi de l'hygiène ne sont pas observées, le pourcentage d'asthmatiques est important, de 1 à 2 % (KOCANE, OHRID, etc.). Au contraire, dans les quartiers modernes aux larges boulevards asphaltés, bordés de rangées d'arbres et de cités modernes avec des parcs et des pelouses, si bien qu'entre les différents batiments l'aération est satisfaisante, on y constate très peu d'asthme (0.5 % dans les nouvelles parties de Belgrade). Il en est de même dans les villages surpeuplés des régions de plaines, où les maisons sont resserrées les unes contre les autres, où, sur de petites parcelles de terrain, les granges, les écuries, la fosse à fumier se trouvent auprès des bâtiments-mêmes d'habitation, et où le pourcentage d'asthme est élevé (1 % et plus) ; par contre, dans les villages de montagnes où les maisons sont

éloignées les unes des autres et entourées de vergers, où l'air est excessivement pur (20 à 100 particules de poussière dans 1 cc d'air, Serbie méridionale), le pourcentage d'asthme est inférieur à 0,04 %.

Le genre de vie se montre aussi de plus en plus comme un facteur important dans l'apparition de l'asthme et des manifestations allergiques. A ce point de vue la différence est nette entre les habitants que vivent la vie moderne des grandes villes, a un rythme accéléré, et que sont exposés à un vacarme perpetuel et à un nombre de plus en plus grand de nouveaux allergènes de leurs vêtements, de leur mobilier, de leurs aliments, des médicaments, des produits de beauté, etc., et où sont de plus en plus fréquentes les distonies du système neuro-végétatif, où l'état psychique est labile, surexcité et déséquilibré, et où l'asthme et les manifestations allergiques sont fréquents; et, contrairement à cela, se manifeste l'absence d'asthme chez les habitants des villages reculés de montagnes où s'écoule encore la vie à un rythme tranquille et lent (Serbie méridionale, Macédoine, etc.).

Ce seraient là les facteurs principaux dans l'apparition de l'asthme, que nos recherches ont mis en évidence, en Yougoslavie. Ils sont déjà par eux-memes complexes si bien qu'il est difficile de les séparer nettement les uns des autres, ce qui du reste n'est pas tellment important car tous ces examens montrent qu'ils agissent ensemble, conjointement, et que l'état d'allergie des habitants d'une localité, d'un milieu, dépend de toutes les conditions asthmogènes favorables de ce milieu, et qu'il n'en est que la résultante.

Le rôle de ces facteurs est encore confirmé par les travaux d'assainissement: amélioration des conditions de travail dans certaines fabriques (pour la préparation des antibiotiques, boulangeries, etc.), solutions urbanistes des agglomérations, installation des égoûts et autres mesures d'hygiène, construction de diques contre les inondations, etc.

A quels moments du mécanisme allergique: sensibilisation, constitution du tissu de choc ou hypersensibilité des effecteurs-influent les facteurs mentionnés il est difficile de le dire. La plupart d'entre eux (infections, conditions géoclimatiques, nature des allergènes, etc.) agissent simultanément sur la majorité des moments d'allergisation mentionnés.

Nous pensons qu'en poursuivant ce travail et en y introduisant des méthodes diagnostiques plus nouvelles, les résultats obtenus seront plus complétés, et contribueront à dévoiler un peu plus cette "mystérieuse maladie".

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FREQUENCY OF AETIOLOGICAL FACTORS IN BRONCHIAL ASTHMA IN AUSTRALIA

P. WARD FARMER. (*)

Australia is a vast island continent of almost three million square miles lying in the southern hemisphere between the Indian and Pacific Oceans. Its northern boundary almost stretches to the Equator while the southern limits extend to the cooler temperate zones bordering on the Southern Ocean. Apart from the tropical areas, where there is a very high annual rainfall, in the south eastern part where most of the population of twelve million dwell there is a moderate average rainfall. Most of the population is concentrated in such cities as Sydney, Melbourne, Brisbane, Adelaide and Perth, that is, around the coast line in the Southern half of the Continent. The rural areas are of course much less densely populated. Most of the central area of Australia is semi-desert, with a low rainfall and is sparsely populated.

It is estimated that at least six hundred thousand persons in Australia have sufferd an attack of asthma at one time or another but the figure may be much higher. Apart from the high rate of incidence symptoms tend to be very severe in many cases.

In Victoria and New South Wales, especially, on or near the coastal areas, marked changes of temperature may occur, the temperature dropping 20 or 30° F. degrees in a few minutes and this undoubtedly aggravates asthma or even seems to initiate attacks in susceptible individuals. In summer humidity tends to be high in the coastal areas especially in New South Wales and Southern Queensland and this appears to make symptoms worse.

Allergic factors - inhalant allergens appear to be the main triggers in starting attacks in at least 70 % of cases in the temperate zones. This is suggested by positive skin test reactions, the results of avoidance of inhalants, and the satisfactory results obtained with specific desensitisation. Apart from animal danders such as cat hair, cattle hair, horse dander etc., which are important in a certain number of cases, "house dust" allergen is the most important perennial inhalant and a very potent strain of this allergen can be obtained from dwellings in Sydney and Melbourne, but particulary in Melbourne. After precipita-

(*) M. B., M. R. C. P., London

tion of the crude extract with acetone and washing with alcohol and aether the resultant material gives very large reactions when tested on the skin of sensitive individuals. Possibly humidity is a factor in producing this most active allergen in these cities. The dust obtained in Adelaide and Perth is not quite so active.

Owing to the temperature and nature of the climate in the southern areas a profuse growth of wild spring grasses occurs in the spring, that is, from about August to December depending on the latitude. The most common grasses found are ryegrass, cocksfoot, kentucky blue, yorkshire fog, wild oats and barley but many others occur. The summer grasses such as couch and paspalum do not seem to cause so much asthma. However, if a heavy rainfall occurs in the late spring this may cause a secondary growth of spring grasses resulting in an upsurge of asthma in summer, that is, in January and February. There is therefore a very high incidence of pollen asthma through the spring with sometimes a prolongation of symptoms into the summer.

Large areas of Victoria and New South Wales are wheat producing and as well crops are grown for pasture on other areas so the pollen from these ripening crops of course swell the quantity of pollen in the atmosphere. Clouds of pollen may be carried many miles especially by south west winds in New South Wales and north winds in Victoria.

Where both house dust and grass pollen are the main precipitating causes patients appear to have their symptoms mainly in the spring and autumn.

Autumn pollens such as those of dahlia, sunflower, chrysanthemum and cosmos do not seem to be an important cause of asthma. More recently ragweed has been observed growing in certain areas of South Australia and New South Wales but has not spread in sufficient quantity to cause symptoms.

Although some allergists in Australia consider that the spores of fungi act as allergens to produce asthma fairly often, most I think would not consider them an important cause compared to inhalants like grass pollens, "house dust" and other epithelia.

Food allergy does seem to be a precipitating cause in a small number of asthma patients especially in children but on the whole would seem to account for a relatively small proportion of attacks.

Infection owing to the cold damp winters in south eastern Australia there is a high incidence of bacterial and viral infections of the respiratory tract in the general population and there is no doubt that these infections account for many cases of asthma in both children and adults. In early childhood up to the age of four or five years, acute infection would appear to be the predominating cause of asthma whereas after this age acquired sensitivities to dust and pollens account for many more attacks. However, infection does cause attacks at any age and asthma associated with hyperplastic sinusitis and secondary infection is observed particularly in patients over forty years of age. Chronic sinus infection associated with asthma does not seem to respond well

to antibiotics whereas when acute infection is present the response is usually good.

In those patients who have exacerbation of symptoms in the autumn when the weather is turning cool it is extremely difficult to tell whether the inhalation of "house dust" due to closing and heating rooms is the important factor or whether acute infection is operating alone as there tends to be an upsurge of respiratory infections at this time of the year.

Drugs.—As in every other country the ingestion of a small quantity of acetylsalicylic acid will precipitate asthma in those sensitive to it and the well known triad of aspirin sensitivity, nasal, polyposis and low blood pressure is not infrequently encountered. Its incidence would appear to be about the same as in other countries.

Asthma due to pecinillin occurs not infrequently and apart from its therapeutic use may affect people from its ingestion in milk and other dairy products. However all penicillin used for mastitis in cattle by dairy farmers must have dye added so that contaminated milk is obvious to the dye.

Asthma resulting from overdosage with allergen extracts of course occurs proportionate to their use and immunization agents such as influenza virus vaccine and poliomyelitis vaccine induce asthma in occasional cases. In my own practice I have encountered several cases of asthma induced by antihistamine drugs.

Psychosomatic effects as a cause of asthma are regarded by general physicians as being much more important than allergists would regard them here and the general consensus of opinion by the members of the Australian Society of Allergists in that they are overrated as a cause.

Apart from so-called "allergic" or "extrinsic" asthma due to the inhalation of air borne allergens and that caused by acute or chronic infection there remain a group of cases (possibly 10 %) who do not fall into one or other or both of these categories. These are the cases whose symptoms usually start after the age of forty years, who give negative skin reactions to allergens, who are likely to have nasal polyposis or hyperplastic sinus disease, and who respond poorly to conventional forms of therapy. Sometimes they improve with a course of very diluted mixed bacterial vaccine suggesting a type of bacterial allergy as the cause. I suppose the proportion of this type of case would be about the same in Australia as in North America and Europe.

Non-specific effects.—The problems of city dust, smog, industrial fumes and tobacco smoke are also important in Australia. However, although smog and exhaust fumes pollute the atmosphere of such cities as Sydney and Melbourne, the concentration of these is not nearly as great as in such large cities as London and Los Angeles as winds carry away fairly quickly these noxious fumes. However, there has been a vast increase of secondary industry in and around the large cities and this will become a real problem in years to come. There is no doubt that

air pollution does affect the health of many asthmatics in Australian cities.

Many millions of cigarettes are still smoked in Australia but this is a world wide problem and not peculiar to Australia.

In general the most important non-specific effects in Australia are climatic, such as rapid changes in temperature and high humidity, city dust and tobacco smoke.

In summary large areas of Australia are being irrigated, forests are being cleared, the intensity and area of crops increasing and the resultant effect is a huge increase in the content of pollen in the atmosphere.

In conclusion I would think that the etiological factors in bronchial asthma in the populated areas of Australia would be very similar to those operating in the temperate zones of North America and Europe.

SYMPOSIUM

DERMOPATIAS ALERGICAS

ALLERGIC DERMOPATHIES

DÉRMOPATHIES ALLÉRGIQUES

ALLERGISCHE HAUTKRANKHEITEN

H. Storck

G. A. Koelsche

J. Gómez Orbaneja

C. D. Calnan

ALLERGIC MECHANISMS IN SKIN DISEASES

H. STORCK.

From the Dermatological Department of the University Medical School Zurich.
Director: Prof. Dr. med. HANS STORCK.

In spite of the obvious accessibility to the skin, only few dermatoses are recognized with probability or certainty as being of allergic nature. As diseases of the delayed reaction type, we may quote v. g. the contact eczema, the eczematous photodermatitis, the skin reactions after fungal and microbial infections (also tuberculids, purpura Schönlein-Henoch a. s. f.); and as diseases of the immediate reaction type: acute urticaria (caused by helminths, by food or by certain drugs), the thrombocytopenic purpura due to Sedormid, etc. But even in these relatively well investigated reactions, an exact knowledge is often missed as to the nature of the haptens, the formation of antigens, the incubation period, the nature of the antibody and its evidence in vitro, the passive transfer to man, the importance of the antigen-antibody complexes, the mechanisms of the cell lesions caused by the antigen-antibody reaction, and the responsible mediator substances. The testing methods at our disposal facilitate the research of the allergen, but they nevertheless need further development and improvement.

In some other dermatoses, for instance in chronic eczema, atopic dermatitis, chronic urticaria, lupus erythematosus, we presume that allergic mechanisms play a role, but here, the primarily and secondarily sensitizing processes and a possible auto-sensitization are not yet satisfactorily elucidated. Adequate in vitro-methods for immuno-pathological investigation, as they exist for immuno-haematology, are still lacking.

In a third large group of skin diseases, such as bullous affections, malignant granulomatoses, collagen diseases (with the exception of lupus erythematosus), tumors, etc., we must admit that we are still at a complete loss whether direct or indirect immunopathological mechanisms are involved or not.

A great number of problems concerning mechanisms, diagnosis and specific treatment of allergic dermatoses are still unsolved. Recent advances in the knowledge of immuno-tolerance and immunological deficiency may possibly be useful in our discipline in the future, and we hope that the reports which will now follow might also bring light into some of our problems.

ETIOLOGY AND PATHOGENESIS OF CHRONIC URTICARIA

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A review of salient articles in which causes of urticaria are discussed reveals a wide and confusing variance of opinion. This is due largely, we believe, to the fact that often in their research on this disease authors fail to differentiate sharply between the acute and the chronic form, but rather treat them as one. In this presentation we are dealing strictly with chronic urticaria, a term that indicates, as we employ it, lesions that have recurred at least once in 4 weeks for a period of 6 months or longer.

WINKELMANN [1] has noted that all urticaria begins as the acute type and that its incidence in the general population has been estimated to range from 10 to 25 per cent. He adds that, conservatively stated, 50 perc ent of patients with urticaria seen in the section of dermatology at the Mayo Clinic have the chronic variety as defined above and alludes to the fact that seldom in such cases can an etiologic diagnosis be made.

Although the most common causes of urticaria and angioedema are allergic reactions to drugs or foods, infections, and psychic factors, in up to 50 per cent of the chronic cases the etiologic agent remains obscure despite the most expert and thorough study, as SHELDON and colleagues[2] have quite properly pointed out. They added that in less than 10 per cent of cases of this type has the etiologic problem been solved by dietary measures.

As a basis for our statistical analysis, we reviewed the case records of 824 patients seen consecutively at the Mayo Clinic during the years 1957 and 1958 in whom a diagnosis of urticaria or angioedema had been made (table 1). Of this group, 236 satisfied our criteria for chronic urticaria and are the basis for the data we shall present.

One hundred twenty-six of the 236 patients were females and 110 were males. The average age at onset of symptoms was 35 years and the average duration of the problem was 6.3 years. One hundred fifty-two patients had urticaria alone, 14 had angioedema alone, and 70 (almost one third) had both at some time during the course of their illness.

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TABLE 1

Urticaria and Angioedema (824 Cases)

	Cases
Acute urticaria (duration less than 6 months) ...	588
Chronic urticaria (duration 6 months or more) ...	236
Males	110
Females	126
Urticaria alone	152
Angioedema alone	14
Both	70

Average age at onset = 35 years.
Average duration = 6.3 years.

For brevity, the term "urticaria" will be used herein to mean urticaria, angioedema, or both.

The records of the patients with chronic urticaria were analyzed as to various etiologic factors involved, both primary and secondary, and as to responses to the programs of treatment used. Each patient had been examined by a consultant in medicine and most of them had been seen also by one or more consultants in dermatology. We defined the etiologic diagnosis as the final consensus of the several consultants as to the most likely cause of the chronic urticaria in any given individual. From this evaluation we found that no definite etiologic factor could be established in 70 per cent of the series, the diagnosis in these cases being chronic urticaria of undetermined cause (table 2). In an additional 22 per cent the etiologic factor was psychogenic. Thus, in only 8 per cent (20 cases) were various allergic of physical factors thought to be specific causes.

The seven cases imputed to physical agents (table 2) included two each of solar, pressure, and factitial urticaria, and one of cold urticaria. The five cases ascribed to foods were due to various commonly irritating

TABLE 2

Etiologic Diagnosis in Chronic Urticaria (236 Cases)

	Cases	
	Number	Per cent
Undetermined	165	70
Psychogenic	51	22
Physical	7	3
Foods	5	2
Drugs	5	2
Cholinergic	3	1

TABLE 3

Contributing Factors (*) in Chronic Urticaria (236 Cases)

	Factors	
	Number	Per cent of 236 cases
Psychogenic	62	26
Foods	38	16
Drugs	37	15
Physical	30	13
Inhalants	4	2
Foci	1	< 1

(*) 172 factors in 106 patients (45 per cent of 236 patients).

edibles including seafood, berries, pork, eggs, tomatoes and citrus fruits. Of the five cases attributed to drugs, four were caused by penicillin. It seems probable that cholinergic urticaria results from exaggerated physiologic response to parasympathetic stimulation.

Having found that the primary etiologic factor was psychogenic or undetermined in 92 per cent of the patients, we analyzed next those factors considered to be of secondary importance (table 3). These were the factors that the dermatologic consultant thought contributed to the chronic urticaria but could not be considered to be the primary agent. They either had caused acute flares of the hives on some occasions but were not consistent in this relationship, or they were known to aggravate the condition when it was present.

TABLE 4

Atopy and Eosinophilia

	Patients	
	Number	Per cent of 236 cases
Atopy:		
Family history of atopy	71	30
Associated allergic diseases	64	27
One or both manifestations of atopy	113	48
Eosinophilia:		
Differential smear reported	150	
Eosinophilia of 5 per cent or greater	23	15 (*)

(*) Per cent of 150 smears.

Psychogenic factors were thought to be a contributory cause of the chronic urticaria in 26 per cent of patients. Remembering that those factors were the primary cause in 22 per cent, we find that emotional factors were implicated etiologically in almost half the patients. Conversely, the same logic reveals that foods, drugs, or other specified factors were each implicated in less than one fifth of the cases.

Two or more contributory agents were noted in a large number of cases. Thus, in 106 patients, or 45 per cent of the group, 172 contributory factors were implicated.

Tabulation of the information on atopy and eosinophilia showed that 30 per cent of the patients gave a family history of allergic diseases including asthma, hay fever and eczema, while 27 per cent gave a personal history of some allergic problem other than urticaria (table 4). Since 113 of the 236 case records reviewed showed a familial or personal history of allergy or both, it is clear that 48 per cent of the patients with chronic urticaria had an atopic constitution.

Although several investigators [3] have failed to find significant eosinophilia in this disease, we evaluated this factor where possible. Of 150 patients who had a differential leukocyte count, 23 had an eosinophilia of 5 per cent or more. In only three of the 23 patients were intestinal parasistes found, and it seemed unlikely that these were responsible in any instance for the urticaria.

The records of the 165 patients in whom the primary etiologic factor was undetermined were analyzed further for possible contributory factors. No factor could be implicated in 79. In the remaining 86, various factors (150 in all) were thought to play only a secondary role etiologically (table 5). As pointed out by other workers [4], multiple factors may trigger the lesions of urticaria, once it has become chronic.

The fact that less than 10 per cent of the patients in this series were found to have a specific allergic or physical basis should help the careful physician to feel more confident rather than uneasy with a diagnosis

TABLE 5

Contributing Factors (*) in Chronic Urticaria of Undetermined Cause (165 Cases — 70 Per Cent of Original Group)

	Factors	
	Number	Per cent of 150 factors
Psychogenic	59	39
Foods	33	22
Drugs	30	20
Physical	23	15
Inhalants	4	3
Foci	1	1

(*) 150 factors in 86 patients (52 per cent of 165 patients).

of chronic urticaria of undetermined etiology, when thorough diagnostic evaluation fails to reveal a definite cause.

The etiologic role of psychogenic factors in chronic urticaria is discussed in an increasing number of articles that appear in the medical literature. STEINHARDT [5] studied the relationship of emotional influences to this condition in a group of patients observed from 1942 to 1947 and in another series treated by him during the period 1948 to 1953. His observations indicated that psychogenic factors were responsible for the chronic urticaria in 15 per cent of the cases in the early series and in 39.5 per cent of those in the later series. He attributed this difference to the physician's increasing awareness of the etiologic importance of emotional stress in this disease.

The exact mechanism of this relatioship has not been elucidated but some useful theories have been advanced. WITTKOWER [6] postulated that this type of chronic urticaria was more prone to develop in persons chronically deprived of affections—who felt frustrated because their basic need was not gratified. SHOEMAKER and colleagues [7] observed that the disease represented a repressive psychologic expression of an unconscious conflict when other mechanisms were inadequate.

In contrast, others [8] have expressed the opinion that psychogenic factors probably never are the primary cause of chronic urticaria. They have theorized that the disease itself causes sufficient aggravation to bring on the emotional conflicts common to these patients.

Of the various etiologic factors considered in our series, the psychogenic were the ones established in the largest number of patients. They were the primary cause in 51 of the 71 cases in which the etiology could be determined (table 2). By contrast, specific tangible agents were established as the prime cause in only 20 patients. Thus, the paucity of patients in whom a definite causative allergic or physical factor could be established is striking. This finding is at variance with those of other workers [8-10].

Further analysis showed that penicillin had been incriminated in 22 of our cases, or 51 per cent of the 42 in which drugs were thought to be either the primary or a contributory factor. Penicillin can and does cause acute urticaria in a person allergic to it, but in the rarer case in which it seems responsible for chronic urticaria it is unclear how this allergic reaction can continue for 6 months or longer without any overt contact with penicillin subsequent to that which set off the original acute condition. One possible explanation is that certain highly sensitive individuals, despite efforts by the government to prevent the use of meat or dairy products from animals treated with penicillin, obtain enough penicillin from these sources to perpetuate their disease [11].

Aspirin was a suspected factor in 15 of the 42 cases in which drugs were implicated etiologically. This frequency may be explained partly by its wide and often indiscriminate use, but we must suspect that the drug is strongly urticariogenic in its own right. WARIN [12] reported that in a group of patients with chronic urticaria studied by him, one third

noted aggravation of their symptoms following the use of aspirin, and the degree was proportional to the dose.

Other drugs or therapeutic agents involved less frequently as causative agents in this series included sulfonamides, tetracyclines, horse serum and vaccines. Rare was the instance in which the drug responsible was a known histamine releaser.

It is well recognized that allergic reactions to foods are prominent in the etiology of acute urticaria. The importance of foods in chronic urticaria, however, is not so easily established, and reports in the literature indicate a wide variation of opinion as to the frequency with which they can be implicated in this disease [9-13].

In our series we were unable to find the common association of food allergy with chronic urticaria reported by some authors. We thought it the primary cause in only five cases, and a contributory factor in 38 others—a total of 43 cases. In most of these (55 per cent), two or more foods were implicated, but in almost all instances the food involved was a member of the same small group, namely strawberries, tomatoes, chocolate, pork, fish, eggs and spices.

To settle the question of food allergy as a cause of chronic urticaria in any given case, our approach is by way of a careful and detailed history, sometimes combined with the use of a modified elimination diet. Experience at our clinic has indicated that in chronic urticaria shown to be due to allergic reactions to foods, certain foods are incriminated most frequently. These include eggs, chocolate, nuts, fish, seafoods, fresh berries, tomatoes, fresh pork, cheese, grapes and bananas. These are excluded from the diets for etiologic study and therapeutic trial. In many instances in which foods were involved, the patients were quite aware of the offenders from prior experience, but either did not know how to avoid the food in all its forms or were not concerned enough about the aggravation of their urticaria to eliminate the food rigidly.

In all cases, chronic urticaria due definitely to allergic reactions to foods was relieved by elimination of the offenders. Since these foods, with the exception of milk, were not essential to an adequate diet, no nutritional deficiency was imposed on the patient by their exclusion. Antihistamines were used frequently as adjuncts to the dietary program, because of their effectiveness in affording symptomatic relief in most instances.

To assess the possibility of food allergy as the cause of chronic urticaria, skin tests rarely are employed at the Mayo Clinic. We believe that correlation between skin reactivity to foods and clinical sensitivity is too poor to justify this type of investigation [14-15]. An added reason for impugning the value of skin testing in such patients is that 18 per cent of the 236 in this series had marked dermatographism. This alone would have invalidated the results of the skin tests in that segment of the total group [16].

Seventy-three of the 236 patients had had extensive skin testing, usually elsewhere before coming to the Mayo Clinic. In all but nine of these patients the skin test had given a positive reaction to one or more substances. Despite this, we were unable to find an instance in which

this type of evaluation had helped elucidate the etiology or aided management of the chronic urticaria.

The pathogenesis of chronic urticaria is believed to be release of histamine from the mast cells into the skin, which causes vasodilatation and increased permeability of the vessel wall, resulting in erythema, edema, and local axon flare [8]. Angioedema occurs if the histamine is liberated in the deeper loose subcutaneous tissues. The exact way in which this substance is set free has not been determined definitely.

The antigen-antibody mechanism for histamine release is the one operative most frequently in other atopic diseases, but seldom is there proof of its action in chronic urticaria [1]. Many agents, including some commonly used drugs, bee and snake venom, egg albumen and, in some instances, strawberries and shellfish, are known to release histamine by some primary action on the skin without the presence of demonstrable [1] antibody. In fact, antibodies are rarely demonstrated in chronic urticaria.

It may be worthwhile to entertain the concept that, in the great majority of patients with this disease, there is some as yet unknown common mediator which, because of its presence in either excessive or deficient amounts consequent to any of a variety of nonspecific stimuli, sets off the chain of events culminating in histamine release and urticaria.

In recent years, much interest has developed in serum permeability factors and their possible relationship to the onset and continuation of urticaria and angioedema. It has been postulated that, in certain individuals, there is a lack of inhibitors of kallikreins [17]. As a result of this deficit, uncontrolled vasodilatation occurs in the skin with consequent urticaria and angioedema.

WINKELMANN [1] has observed that the passage of time diminishes the reaction of chronic urticaria, indicating that the body is capable of controlling this reaction to some extent by itself. When the original flare of urticaria occurs, it is important, he states, to supress this reaction completely and long enough for the body to reject it. He states further that much chronic urticaria could well be avoided if this approach were followed; a minimum of 2 weeks of antihistaminic therapy after subsidence of clinical signs, in his opinion, should go a long way toward achieving this goal.

The final portion of our study relates to the results of the various programs of treatment used elsewhere and subsequently at the Mayo Clinic for the 236 patients with chronic urticaria. Antihistamines gave the best results; 91 per cent of the patients who used them obtained at least some relief (table 6). In contrast, three fourths of the patients treated with elimination diets received no help at all.

Steroids also produced a favorable response in most patients who used them, but the percentage benefited was significantly smaller than the percentage benefited by the use of antihistamines. Most patients treated with steroids received them before coming to the Mayo Clinic, where they are rarely prescribed for chronic urticaria. Since antihistamines afford relief that is as good as or better than that offered by

TABLE 6

Treatment Programs

Treatment	Patients treated		Results of treatment		
	Number	Per cent	Suppression, per cent	Helpful, per cent	No help, per cent
Antihistamines	191	81	39	52	9
Elimination diets	108	46	7	19	74
Steroids	66	28	21	42	37
Sympathomimetics	30	13	40	50	10
Tranquilizers	30	13	3	53	44
Desensitization	26	11		23	77
Other	10	4	50	40	10

steroids in most patients, there seems to be no valid reason for using steroids with their greater potential for undesirable side effects.

Sympathomimetic drugs gave good relief in 90 per cent of patients who took them (table 6), but these were mainly patients with attacks of extensive urticaria or angioedema managed successfully with epinephrine during the acute phase. Patients with refractory chronic urticaria may do well with a combination of antihistamines and ephedrine. Treatment with tranquilizers and desensitization was disappointing, although tranquilizers were used infrequently in the years concerned in this study.

Eliminative measures are to be preferred to desensitization in cases in which inhalant allergens are incriminated etiologically. The patient must avoid ingestion of implicated foods or drugs and inhalation of offending air-borne substances. Foci of infection or intestinal parasites when of etiologic importance must be eradicated.

Conclusion.

Elucidation of the etiology and pathogenesis of chronic urticaria continues to be a challenge to the clinical investigator. Although some basic facts have been established, many observations still demand explanation. When this is forthcoming it may contribute materially to the understanding of the large group of cases of chronic urticaria presently classified as of undetermined etiology. Finally, even though the cause and mechanism of this disease are not well understood, we fortunately have available therapeutic agents, namely antihistamines, that make effective control of the condition possible in most instances.

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PATOGENIA DE LA SENSIBILIDAD EPIDERMICA

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En la clínica observamos manifestaciones eczematosas con todas las características de ese proceso, que son producidas por sustancias de bajo peso molecular y muchas veces de constitución química sencilla, resultado de la acción de dichas sustancias sobre la superficie cutánea. En ese grupo de manifestaciones, unas son consecuencia de la acción de la propia sustancia, de su calidad irritante o tóxica, otras de una actividad que, por determinar, pasado algún tiempo, un aumento de la sensibilidad de la piel a las mismas, consideramos como sensibilizante. La piel reaccionaría en este último caso a una concentración de la sustancia muy inferior a la que es precisa para determinar la sensibilización. Cabe preguntarse qué mecanismo patogénico tiene este cambio reaccional, específico y adquirido, de la piel.

Para que se determine ese cambio es preciso la acción de la sustancia sensibilizante sobre la superficie cutánea a una concentración mínima, variable para cada producto químico, teniendo lugar aun cuando la extensión de la superficie sobre que se aplica sea mínima, y no lográndola aun cuando la extensión sea grande si la concentración es inferior a la precisa.

Pasado un cierto tiempo, período de incubación, variable según la sustancia y los sujetos sensibilizados, la nueva aplicación de la sustancia determina una reacción de carácter eczematoso a concentraciones mucho menores. Esta reacción de carácter tardío se acompaña de una serie de alteraciones histológicas que hoy sirven no sólo para calificar el proceso de la sensibilización en sí mismo, sino para diferenciarlo de la acción, similar desde el punto de vista clínico, que puede tener la acción irritativa o tóxica de esa misma sustancia (por lo general a mayores concentraciones) o de otras sustancias. Aun cuando el acuerdo no es unánime (probablemente debido a la frecuencia con que las sustancias ejercen una doble acción, a la vez sensibilizante y tóxica), caracterizarían a la reacción eczematosa las alteraciones dérmicas, probablemente iniciales, la espongiosis en la epidermis, más tarde la vesiculación, predominante en capas profundas del cuerpo mucoso, y la exocitosis de elementos monucleados, linfocitarios, en contraste con las lesiones vesiculosas subcorneales, con componente leucocitario de tipo polinu-

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clear, y la lisis de elementos celulares epidérmicos que se observan en la reacción a la acción de sustancias tóxicas.

La diferenciación sería difícil de establecer, por las razones dichas, si no se acompañase el fenómeno de la sensibilización de una generalización de la misma; es decir, que la totalidad de la superficie cutánea responde con la manifestación típica a la aplicación de la sustancia sensibilizante, a concentraciones bajas, desprovistas de acción tóxica o irritativa.

Se ha considerado a este fenómeno reaccional como asentado en la capa epidérmica, en contraste con lo que sucede con otras manifestaciones alérgicas, si bien, por lo que hoy sabemos, por muchas razones, el desarrollo de las manifestaciones en esa capa está lejos de conceptuarse como exclusivo, y es muy posible que tenga tan sólo carácter secundario.

Una vez establecida la sensibilidad perduraría toda la vida, o al menos un largo tiempo, en la mayor parte de los casos, aun cuando se hayan señalado casos de remisión espontánea en el curso de pocas semanas.

Esta secuencia, acción externa sobre la piel, período de incubación o de latencia, pasado el cual la piel va a reaccionar bruscamente con la aparición de manifestaciones, que son expresión de un cambio frente a la acción repetida de la sustancia, permitiría en principio a considerar el fenómeno como un proceso de carácter alérgico (con su mismo rasgo específico), ya que la reacción se determina a la sustancia que ejerció la acción primera y no a otras, sin que los fenómenos de sensibilidad cruzada, por comunidad de radicales químicos en su composición, a varias sustancias, ni los fenómenos de polivalencia (menos frecuentes de lo que se había supuesto) lo pongan en duda.

Llamó, sin embargo, la atención, desde un principio, que las sustancias activas no tenían las características de los antígenos conocidos hasta entonces, y que, por otra parte, todos los ensayos encaminados a demostrar la existencia de anticuerpos, en la misma forma que se había logrado en otros procesos, daban resultados negativos. Por otra parte, el tipo de reacción que se producía no tenía las características de las manifestaciones anafilácticas ni la de los procesos alérgicos en que se lograba la transmisión pasiva, ya que la aplicación de la sustancia determinaba una reacción al cabo de un cierto tiempo, más prolongado, lo que hacía hablar de una alergia tardía o retardada. Si queríamos vincular las manifestaciones a un proceso alérgico no había más remedio que referirlas a un tipo distinto que no tendría otra analogía que la reacción tardía o retardada de la sensibilidad tuberculínica, o frente a toxinas o gérmenes bacterianos. Por ello se ha pensado que la sustancia sensibilizante podría determinar en el organismo la formación de otra que, puesta en evidencia, permitiría demostrar que se trataba de un proceso alérgico en sentido estricto, de un conflicto antígeno-anticuerpo.

Con la acción sensibilizante de la sustancia puede coexistir una acción tóxica que facilitaría la primera; aquélla puede lograrse en todos los sujetos con sólo aumentar la concentración de la sustancia activa, aunque no en todos se consigue con la misma facilidad. Habría, pues, sujetos que se sensibilizan fácilmente y otros que lo son con dificultad, lo

que obliga a admitir (aun cuando prescindamos de factores genéticos más evidentes en la distinta capacidad de los diferentes animales para ser sensibilizados en el ensayo experimental) al menos una predisposición variable; serán factores coadyuvantes, aun cuando sean inespecíficos si se quiere, los que la hacen posible y fácil en un número de sujetos.

Añádase a esto el que sujetos que son sensibles a determinadas sustancias, sin embargo, no presentan manifestaciones pese al contacto con las mismas, en los que la sensibilidad tiene un carácter regional, hechos que no tienen fácil explicación, y que nos hablan de la complejidad del problema. Por otra parte sujetos sensibles presentan tan sólo manifestaciones, pese al contacto continuado, en determinadas épocas del año, o en determinadas circunstancias, en probable relación con factores de realización o correlativos que hacen evidente el fenómeno.

El reiterado fracaso de demostrar de manera directa la existencia de anticuerpos, y lograr, salvo excepciones, una transmisión pasiva en esta forma de sensibilidad, ha hecho pensar a algunos que un grupo de las sensibilidades de contacto tengan su asiento en una alteración enzimática (por ejemplo, en la sensibilidad a los metales).

Hay, pues, la necesidad de demostrar la existencia de un mecanismo específico, alérgico, en la sensibilidad de contacto para considerarla como tal, y por ello analizaremos más adelante los resultados obtenidos en la demostración indirecta de las sustancias que ejercen la función de anticuerpos y las lagunas que esos resultados dejan aun para una afirmación rotunda en ese sentido.

Por eso analizamos sucesivamente la calidad de las sustancias antigénicas, las supuestas sustancias reactivas o anticuerpo-similares, el mecanismo de la generalización de la sensibilización, la posibilidad de lograr una transmisión pasiva de la sensibilidad y los factores que contribuyen a que sea posible, así como la predisposición a la misma, y con ello señalamos cuáles son las lagunas en el conocimiento y qué hechos contradictorios observamos en estos estudios.

Sensibilización. Agentes sensibilizantes.

En la sensibilización por contacto se conocen mejor dos grupos de sustancias activas, de fórmula química sencilla y de alto poder sensibilizante, y que son utilizadas con preferencia en los ensayos experimentales. Por un lado, las representadas por el dinitroclorobenzeno, el picril clorid y la prímula, y por otro, sustancias muy simples de carácter metálico, como las sales de cromo, las de níquel, mercurio, etc.

En ellas se ha querido reconocer una afinidad por los componentes tisulares, que se supone es precisa para que la sensibilidad tenga lugar. Actuarían así como haptenos, copulándose a sustancias más complejas (del grupo de las proteínas) y dando lugar con ello a un neoantígeno que determinaría la sensibilidad a la sustancia hapténica. La proteína, o parte de la misma, actuaría como sustancia vectora, sin que la misma fuera precisa en el fenómeno de demostración de la sensibilidad ya producida. Ya en los estudios iniciales se pudo ver cómo la sensibilidad

se hacía a radicales químicos determinados, que, comunes a varias sustancias químicas, explicarían las reacciones cruzadas o de grupo. Serían falsas situaciones de sensibilidad polivalente, diferentes de las sensibilizaciones a distintos compuestos, menos frecuentes, éstas, de lo que se ha considerado hasta ahora.

Las sustancias con capacidad sensibilizante estarían, pues, dotadas de propiedades que explicarían su fácil copulación a elementos celulares o tisulares para formar el antígeno más completo. Así algunas de ellas, como el formol y el cromo, son empleadas en los estudios histológicos en el proceso de fijación de los tejidos; otras tendrían de común su capacidad para desdoblar derivados halogenados en medio alcalino o para unirse a radicales amínicos.

Habría, pues, una diferencia en la fórmula y en el peso molecular de los compuestos que determinan la sensibilidad y de los que la desencadenan una vez establecida. Así, la unión de sustancias químicas sensibilizantes a un aminoácido (por ejemplo, el caso del dinitroclorobenzeno o el del níquel con la alanina) potencia su índice de sensibilización, y, en cambio, su unión a proteínas no es activa (incluso no tienen capacidad de penetración). Estudios de MAYER consideran que la diferencia entre la sensibilidad tardía, del tipo de contacto, con la precoz, puede ser relacionada a la distinta formación del complejo antigénico con proteínas fibrosas en el primer caso, con proteínas globulosas en el segundo.

En la sensibilización a sustancias químicas, especialmente en lo que se refiere a la sensibilización a sustancias de tipo metálico, que cabe considerar algo distinta, no sólo en cuanto a los caracteres morfológicos de las lesiones clínicas, sino también en cuanto a su duración (cronicidad), por la persistencia de las mismas, aun suprimido el contacto (fenómenos cuya supuesta explicación no es el momento de considerar aquí), se ha estudiado la capacidad de penetración de las sustancias en la piel, ya mediante productos marcados con isótopos radiactivos, ya mediante su extracción de la piel, algún tiempo después de su aplicación, no encontrándose diferencias entre los sujetos sensibilizados y no sensibilizados. Sin embargo, en algunos estudios se ha atribuído la diferente capacidad sensibilizante entre el Cr. III y el Cr. VI a su distinto poder de penetración, y con ello a la posibilidad de alcanzar los extractos epidérmicos más profundos, aquellos en los que se presentan inicial y más marcadamente las lesiones en el eczema de contacto. Respecto al contenido en cromo después de su aplicación sobre la superficie cutánea, unos estudios señalan que es el mismo y otros que es menor en el sujeto sensibilizado que en el normal, aun cuando una extracción del cromo no conjugado indicaría en estos últimos casos que el conjugado es el mismo (de ahí la posibilidad de que el no conjugado juegue un papel en la sensibilización, así como el reconocimiento hecho recientemente de que también el cromo Cr. III tiene capacidad sensibilizante.

Actúe de una manera u otra la sustancia sensibilizante, y sea ésta la propia aplicada sobre la piel o la resultante de su acción sobre la superficie cutánea (sustancia resultado de una copulación), el hecho es que al cabo de un tiempo después de una aplicación o de varias (acom-

pañado o no de un fenómeno de "aufflammerung") se demuestra la sensibilidad. Esta sensibilización tendría después de ese tiempo un carácter generalizado.

Pero los hechos no son tan sencillos, puesto que se ha demostrado que los sujetos sensibilizados no dan tan sólo una prueba de contacto positiva, sino que también reaccionan a la prueba intradérmica. Esta prueba intradérmica (observada con compuestos metálicos como con otros compuestos) es específica, y más sensible que la epicutánea, ya que un cierto número de casos dan positiva la primera y no la segunda. No habría, por otra parte, en la práctica de esta prueba el peligro de determinar una sensibilización activa. Se interpreta ese resultado en concordancia con la idea de que la sensibilidad también afectaría a la dermis, supuesto de acuerdo con la existencia siempre de lesiones histológicas dérmicas, consideradas como las más precoces. Las lesiones epidérmicas serían en ese caso secundarias, más o menos marcadas según la forma de aplicación de la sustancia, pero siempre resultado de una sensibilidad dérmica primaria, o al menos de una reacción de la dermis. También se ha interpretado esa reacción positiva dérmica como expresión de una doble sensibilidad, dérmica y epicutánea, que pueden coexistir. La sensibilidad dérmica tendría también carácter tardío, como se ha comprobado reiteradamente, y sería similar a la sensibilidad tuberculínica.

Estos hechos están en concordancia con la falta de acción citotóxica directa del antígeno sobre las células epidérmicas sensibilizadas y con la necesidad demostrada de que la epidermis esté unida a la dermis para que se presenten las alteraciones características de la reacción de la hipersensibilidad. Sabemos, además, que la unión *in vitro* del antígeno y de las sustancias tisulares no tienen capacidad sensibilizante, y que es precisa su unión o copulación *in vivo*.

Nosotros hemos observado algún hecho más, después de comprobar la sensibilidad por la vía intradérmica, y su negatividad en los testigos, y es que si en esos sujetos sensibles, con pruebas epicutáneas y dérmicas positivas, inyectamos el antígeno adicionado *in vitro* con extractos epidérmicos, esa positividad a la inyección intradérmica no se produce, queda anulada, y se hace positiva en esas mismas condiciones si la inyección se practica en un lugar en que anteriormente fueron inyectadas suspensiones de leucocitos de procedencia sanguínea. Habría, pues, en la reacción a la prueba intradérmica, en sujeto sensibilizado, una posibilidad de neutralización de la misma por adición al antígeno de células epidérmicas, y por otro lado, su positivización, en esas condiciones, en presencia de leucocitos.

Que esas pruebas intradérmicas no son resultado de una acción tóxica ni que determinan una sensibilización activa, lo demuestra que en los testigos, por un lado, son negativas, y que su ejecución no determina un fenómeno de sensibilización, controlado varias semanas después.

Estas diferentes reacciones, localizadas en la dermis o en la epidermis, estarían condicionadas, para algunos, por el distinto carácter de la sustancia vectora (Schlepper), que de esa manera dejaría de ser indiferente y matizaría la reacción, en tanto que para otros sería tan

sólo el órgano de la reacción, el órgano del choque, el determinante de la diferencia.

Siguiendo otras vías es más difícil la sensibilización; ya hemos señalado un resultado comprobatorio de lo afirmado ya hace tiempo: la dificultad de la sensibilización por vía intradérmica, así como por las vías ganglionar, subcutánea o venosa (aun cuando con las primeras es posible). Muy conocido y comprobado es el resultado de la sensibilización por la vía peritoneal cuando el antígeno es inyectado conjuntamente con coadyuvantes, como el de FREUND, por ejemplo.

Podría concluirse de estos hechos que la sensibilidad epidérmica se logra por acción de las sustancias *in vivo* sobre la epidermis, precisándose de la unión de éstas a la dermis. Que es precisa una copulación con determinadas sustancias, ya que con otras el tipo de sensibilidad sería distinto. Que sería preciso, cualquiera que sea la vía de la introducción del antígeno, que llegue a determinados niveles de la epidermis para que se presente el fenómeno.

Que existiría una sensibilidad dérmica tardía, diferente o idéntica a la epicutánea, que daría una prueba negativa con la mezcla del antígeno con elementos tisulares y que en cambio se positiviza de nuevo en presencia de leucocitos sanguíneos.

Generalización de la sensibilidad.

Si la sensibilidad que se determina por la aplicación del antígeno quedase limitada a la zona de acción del mismo, los problemas serían muy distintos de los que plantea su generalización, es decir, la determinación de una capacidad de reacción de la totalidad de la superficie cutánea. Ya hemos dicho que en algunos casos, reducidos, esa sensibilidad es más manifiesta o exclusiva en una zona limitada de la superficie cutánea, fenómeno o resultado que no tiene fácil explicación en el conjunto de nuestros conocimientos.

En un principio, dada la consideración de que la sensibilidad era epidérmica, se pensó que se propagaba por contigüidad, de unas células epidérmicas a otras. Ensayos sucesivos, a veces contradictorios, han venido a demostrar, de manera indudable, que esto no sucede así. La incisión circular de la piel alrededor del punto de aplicación de la sustancia antigénica, cuando es suficientemente profunda, impide el fenómeno de la generalización de la sensibilidad. El intento de sensibilizar sobre un islote cutáneo, unido al resto del organismo únicamente por un pedículo vasculonervioso, no tiene resultado. Este hecho experimental no estaría en contradicción con la concepción primitiva, la de la propagación por contigüidad, pero se anula ese supuesto con la demostración de que la aplicación de la sustancia sensibilizante sobre cualquier punto del resto de la superficie cutánea determina la sensibilización del islote cutáneo, preparado en la forma mencionada. El aislamiento, por tanto, de una zona de piel libre de sus conexiones epidérmicas con las células próximas no impide que llegue a ser sensible.

Cuando el islote cutáneo queda unido al resto de la piel por un puente cutáneo, la sensibilización generalizada tiene lugar por la aplicación del antígeno sobre aquél. La diferencia en la preparación técnica en este caso, respecto al anterior, sería la conservación de algunas conexiones linfáticas. Esa propagación tendría lugar a través de ellas, lo que se confirmaría por la experiencia de que extirpados los ganglios regionales correspondientes a la zona que es sometida a la experiencia, no se presenta la sensibilización, si la intervención es realizada en los primeros días, y lo hace en un número creciente de casos a medida que se retarda ese momento. La sensibilidad, por tanto, tendría lugar con la participacicón del sistema linfático.

Que la sensibilización no se produce en la superficie cutánea, sino que está condicionada por un factor reactivo que procede del resto del organismo, y que debe llegar a la piel, lo demostrarían las experiencias de injertos en animales gemelos y en sujetos gemelos univitelinos; única circunstancia en que puede esperarse que la piel injertada no sea eliminada. Entonces se observa que la piel trasplantada se comporta inmunológicamente, frente a la sensibilidad, de la misma manera que la piel del receptor. Si un trozo de piel de un animal u hombre donador, reactiva, y procedente, por tanto, de sensibilizado, es trasplantada a un animal o sujeto normal, no sensible, pierde su sensibilidad en días sucesivos. Por lo contrario, la piel de un sujeto normal trasplantada a uno sensible, reacciona a la aplicación sobre ella de la sustancia antigénica. No residiría por tanto en las células epidérmicas la sensibilidad, sino que teniendo lugar en las mismas una reacción, sería debida a la aportación de un factor que la condiciona y procedente del sujeto sensible.

Estos hechos quedan comprobados con el comportamiento de los animales parabiosados, ya por conexión entre distintos órganos, ya por sutura cutánea; el animal no sensibilizado participa en este caso de la reacción frente a la sustancia sensibilizante del animal emparejado. Una vez suprimida la parabiosis, continúa reaccionando durante unos días, para agotarse más tarde esa capacidad de reacción.

Factor de reacción (anticuerpo-like).

La producción de una sensibilidad que no queda limitada a la zona de aplicación de la sustancia, sino que se generaliza y la posibilidad de la transmisión pasiva de esa sensibilidad (aun cuando se haya obtenido en un número limitado de casos, como veremos después) hace pensar en la producción de una sustancia que tenga las características de los anticuerpos.

Su investigación *in vitro* en el suero, ya mediante pruebas de fijación del complemento, mediante la aglutinación o la hemolisis, ha dado resultados siempre negativos, aun cuando recientemente algunos autores hayan demostrado que en casos de sensibilidad al cromo, frente a su agregación a los glóbulos rojos, pueda demostrarse *in vitro* la existencia de sustancias reactivas circulantes.

El ensayo experimental de sensibilización por vía intraperitoneal frente a cuerpos químicos de bajo peso molecular, con la adición de un coadyuvante (teoría hapténica) determina la aparición de una sensibilidad generalizada que permite, como veremos después, demostrar que la sustancia activa, aun cuando esa demostración sea indirecta, reside o está vinculada a los linfocitos obtenidos en el exudado peritoneal. Sólo se demostrarían sustancias activas en el suero cuando simultáneamente se produce una sensibilidad de tipo precoz. De la misma manera se demuestra en las células linfáticas de ganglios, bazo y timo esa sustancia activa condicionadora de la sensibilidad tardía del tipo de contacto. De ahí que, como veremos, todos los ensayos se han dirigido a demostrar la posibilidad de la transmisión pasiva utilizando las células linfocitarias.

Otros argumentos en favor de estas afirmaciones se podrían encontrar en el conocimiento histológico de la infiltración y exocitosis en la epidermis, en la típica lesión ezcematosa, por linfocitos, pese al carácter agudo o subagudo de la reacción, así como por el asiento de las lesiones principales en las zonas más profundas de la epidermis, en contraste con las más superficiales que se producen en la acción irritativa o tóxica, inespecíficas, sobre la piel. También en la participación de los ganglios en los casos de sensibilización, con comprobación de alteraciones histológicas, cabe encontrar otro argumento.

Puede agregarse a ello la afirmación de algunos de que la inyección de leucocitos potencia el fenómeno reaccional epidérmico, así como la demostración de la distinta calidad entre los linfocitos en el animal sensibilizador y no sensibilizado, en el cultivo.

Interés en este mismo sentido tienen los intentos de desensibilización específica por la aplicación sobre la superficie cutánea, o por inyección intravenosa, del compuesto químico, ya que en uno y otro caso se consigue reducir o anular la sensibilización, sin que se pueda decir si en el segundo caso se determina por saturación del anticuerpo o por producción de anticuerpos bloqueantes.

Las células linfocitarias de sujeto o animal sensibilizado captarían mayor cantidad de antígeno *in vitro,* según se demuestra con uno marcado con isótopos radiactivos. Los linfocitos en ese caso se lisan con más facilidad *in vitro,* y aumentan durante la fase de sensibilización, según otras experiencias.

Transmisión pasiva de la sensibilidad.

El lograr la transmisión pasiva de la sensibilidad sería la prueba concluyente de su vinculación a un mecanismo alérgico específico. Dado que no ha sido demostrada la existencia de anticuerpos séricos, salvo las aisladas afirmaciones mencionadas, todas las investigaciones se han dirigido a su demostración, aun cuando sea con pruebas indirectas, en los elementos del sistema linfático, que se han demostrado como precisos en la producción del fenómeno de la sensibilización.

Se trata, sin embargo, en el intento de demostrar una transmisión pasiva a la sensibilidad de contacto, de diferenciarla de una sensibilización activa que se haya podido producir por ser el sustrato de la transmisión portador de un principio activo de sensibilización. Es ésta una de las principales objeciones que se han hecho a muchos de los ensayos que se han considerado como positivos en la transmisión pasiva. Incluso es preciso prescindir de una prueba previa en el probando (para seguridad de que no estaba ya sensibilizado) dado que su ejecución puede inducir a una sensibilización, que se podría haber considerado como resultado válido de la segunda experiencia.

El primer ensayo logrado fue el de LANDSTEINER, al sensibilizar al cobaya por vía peritoneal, con un conjugado de la sustancia química, con estromas eritrocíticos y bacilos tuberculosos, y demostrar que el exudado peritoneal de esos animales era capaz de transmitir pasivamente la sensibilidad a otros animales.

Este mismo resultado se obtuvo utilizando bazo, timo o ganglios siempre que las células linfocitarias no estuviesen alteradas, ya que se demostró que su calentamiento podía hacerlo fracasar. Era preciso para lograrlo una cantidad mínima de células. Todas las experiencias se hicieron por vía peritoneal, ya que por otras vías no se obtenían resultados positivos, y en algunos conseguidos con el empleo de la vía intradérmica se objetó que los elementos celulares eran vectores de un factor sensibilizante activo.

Esos resultados y la modalidad de la prueba positiva equipararon la sensibilidad de contacto a compuestos químicos de bajo peso molecular, a la sensibilidad tuberculínica, si bien en ésta se conseguía un resultado positivo en inyección intradérmica o por otras vías.

Ensayos en el hombre, realizados por EPSTEIN, demostraban más tarde la positividad de la transmisión por vía intradérmica para la sensibilidad de contacto, pero era preciso que el número de células empleadas en la transmisión fuese una cantidad elevada, que el alergeno tuviera un alto poder sensibilizante, que hubiese determinado un alto grado de sensibilidad y que el animal receptor fuese susceptible de una sensibilización activa (calidad inmunitaria del mismo), así como que las células empleadas no hubiesen sufrido alteración alguna. Condición esta última que contrastaba con la posibilidad de transmitir la sensibilidad tuberculínica pasivamente con un eluído de linfocitos (LAWRENCE).

La sensibilidad transmitida de esa forma tendría tres modalidades de reacción: su agotamiento al cabo de unas semanas, su persistencia durante un tiempo prolongado, y una tercera modalidad bifásica, en que, agotada la sensibilidad en su primera fase, volvía a reaparecer y persistir durante algún tiempo. En estos dos últimos casos, difíciles de explicar, no se consigue anular la objeción (aun cuando se haya prescindido de tests anteriores en los receptores) de que puede tratarse de una sensibilización activa, determinada por algún factor incluido en los linfocitos inoculados.

Ya hemos señalado cómo el papel que juegan los linfocitos estaba corroborado por su presencia en las alteraciones histológicas, en la reac-

ción eczematosa, por las alteraciones ganglionares y por hechos experimentales más recientes, como es su distinto comportamiento según el animal sea sensible o no, por su desaparición rápida en el animal sensible, en tanto en el animal normal se acumulan en el bazo y en el hígado (lo que se logra evidenciar por estar marcados con sustancias radiactivas), técnica que permite también demostrar su acumulación en la zona de aplicación del antígeno. En todo caso, en la transmisión pasiva de la sensibilidad se trata de un fenómeno específico en relación con una estructura química determinada.

El distinto resultado de los ensayos de transmisión pasiva puede explicarse, en el caso de su fracaso, porque no exista el factor transferencia (anticuerpo like), porque liberada esa sustancia no sea activa pasado algún tiempo (importancia de este factor en la realización de la transmisión pasiva) o por pérdida del mismo por agotamiento. En contrapartida, los resultados positivos, que hicieran creer en una transmisión pasiva lograda, se podrían justificar por producirse una sensibilización activa, o por la acción de un factor inespecífico que exaltara la reacción frente al antígeno.

Por eso tienen interés los estudios de las alteraciones histológicas de las reacciones obtenidas en la transmisión pasiva; en unos se refiere que esas alteraciones son equivalentes a las observadas en la acción tóxica de las sustancias (¡!) y en otros que las alteraciones son esencialmente dérmicas, dato este último que viene a corroborar ideas más recientes, como hemos dicho, del mecanismo de la sensibilidad de contacto.

La participación del sistema linfático quedó demostrada por los ensayos de FREY, si bien es discutible cuál es su función en la formación del factor transferencia: si se produce en el mismo, en los ganglios; si permitiendo éstos el paso de la sustancia antigénica se produciría en otros lugares, o si formada en los ganglios una nueva sustancia ésta sería la inductora de su formación en otros lugares, explicándose así el hecho de que la extirpación ganglionar, diez días más tarde de realizada la acción sensibilizante, no detiene la producción de la sustancia determinante de la sensibilización. En referencia a este punto, se ha querido hacer jugar un papel al sistema nervioso, de lo que nos ocuparemos más adelante.

Como sabemos, la sensibilidad no lo es sólo a la prueba epicutánea, sino que también se demuestra una sensibilidad dérmica, que en algunos casos, como en las sensibilidades a metales, es más constante. En este caso es también posible obtener una transmisión pasiva a esa sensibilidad, en tanto la ejecución simultánea de las pruebas con antígeno solo o de antígeno con inyección previa de leucocitos, realizadas a la vez en el probando, nos han dado un resultado negativo de la primera y positivo de la segunda, salvando de esa manera las objeciones de que sea esta última producto de una acción tóxica o de una sensibilidad previa de tipo activo. La reacción tiene un carácter tardío equivalente a la reacción tuberculínica. Ya HAXTHAUSEN obtuvo un resultado positivo a la transmisión por vía intradérmica, pero lo interpretó como resultado de una sensibilización activa, ya que la refirió a una generalización de la sensibilidad. Recientemente, MENEGHINI ha intentado transmisiones pasivas por in-

yección intradérmica y patch test, con resultado negativo; el antígeno lo aplica mezclado con suero. En nuestros ensayos, cuando inyectamos una mezcla de antígeno y epidermis, hecha previamente, en el lugar en que han sido depositados los linfocitos, vemos que la reacción no tiene lugar, sin que tengamos una explicación para ello. Esa mezcla utilizada pensando que empleábamos el antígeno más completo, formado por la conjugación de esos elementos, impide o hace negativa la prueba que antes era positiva con la aplicación del antígeno metálico sobre la inyección previa de leucocitos (ya hemos señalado cómo de la misma manera se negativiza la prueba del antígeno en inyección intradérmica con esa mezcla en el sujeto sensible).

Tenemos, pues, que existe un factor transmisible contenido en los linfocitos que ejercería la función del anticuerpo en los casos de sensibilidad epicutánea o dérmica de tipo retardado, frente a sustancias químicas de peso molecular bajo. Que ese factor es vectado a la piel, ya que no se encontraría en la misma. Que la piel sí parece ejercer una función en tanto presta a la sustancia sensibilizante una capacidad de sensibilización en relación con su acción sobre la epidermis. Que la inyección intradérmica (positiva en el sensibilizado), sin embargo, no determina por lo común una sensibilización de tipo tardío en el probando normal, como lo prueban la inocuidad en este sentido de las inyecciones, y la falta de una sensibilidad epidérmica en dichos sujetos, ya que la prueba epicutánea es también negativa semanas más tarde.

Factores de realización.

No se suele considerar a la sensibilización de contacto como una atopía, dada la posibilidad de que todo sujeto pueda ser sensibilizado. De ahí se ha pasado a pensar que todo el problema está en la reacción o conflicto entre la sustancia sensibilizante y la producida con los caracteres similares a los anticuerpos. Sin embargo, se conocen estados de sensibilidad epidérmica que no se acompañan de manifestaciones clínicas, pese a que no cabe excluir el contacto con las sustancias activas. Sabemos también que si muchos sujetos se sensibilizan con facilidad, otros, en cambio, lo son muy difícilmente. Además, y desde un punto de vista experimental, conocemos animales que nos son útiles en los ensayos por su posibilidad de sensibilización, en tanto que otros no pueden ser empleados por su total resistencia. De ello puede deducirse que aun cuando hagamos asentar el núcleo de la sensibilidad epidérmica en una reacción de tipo alérgico, ésta se halla condicionada en su realización por distintos factores, unos dependientes de la calidad, modo de acción y concentración de la sustancia sensibilizante, otros relacionados con características determinadas de los sujetos que pueden sufrir la sensibilización y que pueden ser simplemente adjetivos y funcionales, o ya de carácter constitucional. Sería, pues, preciso analizar sistemáticamente cuáles son esos factores de realización que hacen posible que la sensibilidad tenga lugar, y que se produzca el fenómeno de la reacción. Lo mismo cabe decir, aunque en menor grado, respecto a la transmisión

pasiva, sólo realizable en animales de ciertas características inmunológicas.

Hemos hecho ya mención a los factores referibles a la acción de la sustancia sensibiilzante, a su calidad, a la necesidad de que actúe a una concentración determinada, a sus condiciones físicas de solubilidad, a su acción irritativa o tóxica simultánea (posibilidad de obtener una sensibilización en todos los sujetos aumentando la concentración de la sustancia, lo que estaría algunas veces en función de su acción irritativa, que facilita la sensibilización).

Por parte del organismo, las características anatómicas de la superficie cutánea, el mayor o menor grado de espesor de la capa córnea (lo que es evidente en el distinto grado de labilidad, frente a la sensibilización, de las distintas zonas de la piel, según su espesor), la mayor facilidad cuando esa capa está lesionada por acciones mecánicas (raspado, lijado, etc.) o por acciones químicas, condicionan al menos la mayor o menor facilidad para la sensibilización. Pero también juegan un papel en este sentido las condiciones fisiológicas de la cubierta cutánea, su poder más o menos marcado, según los sujetos, para neutralizar la acción de las sustancias alcalinas, aplicadas sobre la misma; su mayor o menor contenido en agua y grasas, lo que influye según las características de solubilidad de las sustancias activas, en su poder de penetración y con ello de sensibilización.

Añádase a esto la acción que puede ejercer la existencia de determinados trastornos hormonales, o la calidad de las diferentes dietas, así como de las influencias climáticas o estacionales. La influencia de carácter inespecífico de las alteraciones nerviosas, en tanto las secciones de los nervios cerebroespinales, o del simpático, no impiden el fenómeno de la sensibilización (ya sabemos que no se produce la sensibilización a partir del islote cutáneo, aun cuando conserve sus relaciones nerviosas, en tanto le faltan las linfáticas). Hay una influencia de los estimulantes o depresores nerviosos sobre la reacción, sobre el fenómeno reaccional y no sobre su producción. En clínica es conocido el hecho de la intensificación y puesta en evidencia de los síntomas por influjos neuropsíquicos o psicosomáticos en el ezcema. El sistema nervioso no jugaría papel alguno como vía centrípeta o centrífuga en la sensibilización.

Que las condiciones del sujeto tienen un valor se demostró por MIESCHER incluso frente a la acción de las sustancias tóxicas sobre la superficie cutánea, ya que su dintel de acción sería también distinto de unos sujetos a otros; no habría, pues, una acción tóxica uniforme.

Y con ello se plantea la existencia de factores de carácter genético que puedan hacer posible la sensibilización o que su realización sea más fácil o difícil. Ya hemos citado el distinto comportamiento de los diferentes animales a este respecto, expresión de unas características constitucionales de tipo inmunológico. Incluso pueden obtenerse camadas de cobayas sensibles o resistentes a la sensibilización. Ya en el hombre se advierten esas diferencias según las razas, así como la que ejerce el distinto estado del sistema reticuloendotelial, evidenciable en procesos patológicos que afectan al mismo.

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AUTOSENSITIZATION AND SKIN DISEASES

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The idea of autoimmunisation to a skin antigen is not a new one. Dr. Arthur WHITFIELD (1921) put forward such a suggestion to explain three observed phenomena in the skin, two being in regard to the spread of eczema. The first was that an area of eczema, such as frequently occurs on the lower leg, may spread extensively in a symmetrically disseminated pattern over the arms, head, neck and trunk, whether in response to trauma, or as an adverse reaction to local applications, or spontaneously. The second was that when the serous fluid from an area of acute exudative eczema is allowed to flow over previously unaffected areas of skin the latter may after a short interval exhibit eczema; and the third was a papular and urticarial eruption following traumatic haematomata in the skin. All of these phenomena are widely accepted as clinically observable facts, but there is no agreement as to their interpretation.

The distinction between cutaneous and systemic diseases has become increasingly blurred in recent years and it seems likely that only traumatic, infective, congenital, neoplastic and some degenerative disorders will be regarded as purely cutaneous. A high proportion of the other dermatoses, many of whose aetiology is unknown, such as eczema, lichen planus, psoriasis, dermatitis herpetiformis and so on, are almost certainly systemic affections. Autoimmunity is currently the most fashionable concept of their pathogenesis. Certain criteria have been laid down by WITEBSKY et. al. (1947) for the establishment of autosensitization, while those of WAKSMAN (1959) are even more rigid. One may say at once that these criteria have not been satisfied in relation to any single skin disease, even though the list of possible autoimmune dermatoses is quite a long one - including lupus erythematosus, scleroderma, dermatomyositis, polyarteritis nodosa, eczema, urticaria, psoriasis, pyoderma gangrenosum, allergic vasculitis, chronic anular erythemas, alopecia areata, vitiligo and some of the bullous diseases. I shall purposely omit any discussion of lupus erythematosus and the related so-called collagen diseases because this subject has been so extensively covered

in the medical literature, although I shall refer to some work on dermatomyositis.

Eczema

Some attempts to provide evidence of auto-sensitization in the types of eczema patients described by WHITFIELD, have been made by TEMPLETON et al. (1949) and CORMIA and ESPLIN (1950). Their experiments consisted of skin tests with autologous serum, leukocytes and skin extracts. The latter were either human dander or aqueous extracts of ether washed scales. Equal proportions of immediate and delayed type reactions were obtained, the percentage of positive reactions varying from 20 to 80 per cent (ESPLIN and CORMIA, 1951). The antigen, however, was not characterised and its "purity" remains in doubt.

Evidence that antibody to skin can be produced has been given by HECHT et. al. (1943) and VOISIN and MAURER (1955) in rabbits, by PARISH (1961) in pigs, and by ROSENTHAL et al. (1958) and WILHELMJ et al. (1962) in guinea-pigs. The latter authors found that sensitization was associated with damage to skin and hair. Occasional positive reactions have been obtained in dermatitic swin with fluorescein labelled globulin staining.

A fresh approach from the immunological point of view has now been made by PARISH (1961). He defines autosensitization as "sensitization of the body, by circulating antibody, or delayed cellular type reaction, or both, to constituents of its own tissues". The antibody or cellular agent is cytotoxic, although it could possibly passively sensitize skin cells, which later react to antigen (perhaps liberated from damaged skin). The problem was to isolate an antigen or antibody. This was achieved in four out of eighty one patients with exfoliative dermatitis or widespread dermatitis by PARISH, ROOK and CHAMPION (to be published). The serum antibody could only be demonstrated during periods of active spread of dermatitis. This antibody would agglutinate particles coated with skin extract, and was cytotoxic to human epidermal cells. The agglutinating effect but not the cytotoxication was inactivated by heating to 56°C. The agglutinating antibody could be absorbed out by epidermal cells. The antibody produced a wheal and flare in both the patients and in normal individuals. The cytotoxic effect could be demonstrated on fresh sections and tissue cultured cells. The antibody is present in the gamma-globulin fraction, is inactive at 4°C. and requires the presence of complement. These studies appear to be fairly conclusive evidence of the presence of an autoantibody to skin antigen, but the positive findings in only 4 out of 81 patients suggest that it is unlikely to be the causal mechanism of the generalization of ezcema.

Urticaria

SHELLEY and FLORENCE (1951) described some interesting findings in a patient with intractable urticaria and a chronic mycotic infection associated with sensitivity to trichophytin and other fungal antigens. They were able to show that the patient's red blood cells were coated with the fungal antigen, and that these cells produced a positive immediate reaction in the skin. The urticaria was controlled by continued treatment of the fungal infection. JADASSOHN (1961) had seeen a similar patient. Many antigens can be coated on to red cells in this way, bus such reactions are not genuinely autoimmune.

Fluorescein labelled globulin has also been detected in the lesions of at least one case of 'spontaneous' urticaria lesions, although it may have been associated with lupus ehythematosus, an accepted autoimmune disorder known to be sometimes associated with urticaria. Although the cause in a high proportion of patients with urticaria is not identified, it is likely that non-immunologic mechanisms form their basis, although a small number may be autoimmune (CALNAN, to be published).

Pyoderma gangrenosum

It has long been suspected that ulcerative colitis could be an autoimmune disorder. LONG et al. (1964) have recently investigated two patients with pyoderma grangrenosum and ulcerative colitis, one of whom had hypogammaglobulinemia. They showed delayed hypersensitivity to the intradermal injection of their own leukocytes, although the proportion of lymphocytes in the dose is not given. Autogenous skin grafts were rejected, but such rejection did not occur if the graft was previously rinsed in hydrocortisone acetate. These experiments, although interesting, cannot be regarded as conclusive. In our department, one similar patient investigated to date did not show any dermal sensitivity to her own lymphocytes (TURK, personal communication 1964).

There are many patients with a condition indistinguishable from pyoderma gangrenosum, but who never have any evidence of colitis. Since the condition is not infective and is steroid responsive it would be worth pursuing an autoimmune basis.

Juvenile cirrhosis

It is now believed that juvenille hepatic cirrhosis (also known as lupoid hepatitis, plasma cell hepatitis or active chronic hepatitis) is an autoimmune disorder. It is not infrequently seen in association with ulcerative colitis which is thought to be similar aetiology.

SARKANY (to be published) has recently drawn attention to the as-

sociation of certain skin lesions with this disease. The eruption consists of scanty or widespread inflammatory popules which become haemorrhagic and crusted, and finally resolve leaving small white depressed scars. Histologically, the epidermis contains a crater filled with a plug of hyperkeratosis. The dermo-epidermal junction shows patches of fibrinous material, which is also present round the capillaries. There is a moderate infiltrate in the upper dermis round the small vessels, consisting of lymphocytes, histiocytes and eosinophil leukocytes. Laboratory findings include hypergammaglobulinemia, eosinophilia and the presence of antinuclear auto-antibodies.

SARKANY considers that these skin lesions are a replica of the process which is occurring in the liver, which usually shows a heavy infiltration of the portal spaces by mononuclear cells, histiocytes and eosinophils, as well as parenchymal degenerative changes.

Chronic annular erythemas

The aetiology of most cases and types of the so-called chronic annular erythemas is quite obscure. But there are three clinical associations wich have suggested that they may well be hypersensitivity phenomena. One is their relationship to fungal infections and trichophytin sensitivity, of which there have been many reports in the literature. The second is the association with internal malignant disease; and the third is with an auto-immne reaction to breast secretion. GAMEL (1952) first suggested this in a patient with carcinoma of the breast, who had a widespread eruption which he labelled erythema gyratum repens. The eruption cleared after surgical removal of the cancer. Gold's patient with bronchogenic carcinoma, reported by SCHNEEWEIS (1959) had an identical eruption, which also cleared after X-irradiation and involution of the tumour. More recently, GOLD presented a further case (1964) in whom the skin eruption completely cleared after surgical excision of a carcinoma of the lung. Similar cases have been described by PURDY (1959) DUPERRAT et al. (1961), VAN DIJK (1961) and WOERDEMAN (1964).

Of greater interest, however, is the patient described by SHELLEY and HURLEY (1960). She was a woman of 28 with a five year history of a universal spreading gyrate erythema of the same type, associated with progressive cystic adenomatous hypertrophy of the breasts and generalised hyperpigmentation. The eruption was pruritic and with an urticarial border. Laboratory studies showed a blood eosiniphilia of 32 %, a sedimentation rate of 32 mm./1 hour, hypergammaglobulinaemia (2.41 mg. per cent) as well as a positive L. E. cell test and a false positive serology test for syphilis. Furthermore, they were able to demonstrate by means of the tanned red cell agglutination technique a specific, circulating auto-antibody to the material from the cystic breast tissue. Normal patients did not show this antibody, nor would extracts of normal breast tissue react with it. Surgical removal of almost all the hypertrophic breast tissue resulted in considerable amelioration of the

eruption, and the residual element was controlled with steroids. All the laboratory changes reverted to normal except for persistence of the auto-antibody to the breast tissue.

Dermatomyositis

Although dermatoyositis does not have a special predilection for any particular age group, being encountered equally as often in children as adults, it is now appreciated that when the disease begins in a person over forty years of age, there is a strong suspicion that it will be associated with internal carcinoma. It is even suggested that this will be found in every instance after middle life if a meticulous autopsy is carried out. CURTIS et al. (1952) first suggested that the relationship between the cancer and dermatomyositis might be immunological. Dermatomyositis may remit with ablation of the growth and relapse with its recurrence, and hence may be dependant on some tumour product. The most striking facts, however, are that the tumour precedes the dermatomyositis and that the cancer is almost invariably fatal eventually.

GRACE and DAO (1959) studied a patient with dermatomyositis and carcinoma of the breast. An aqueous extracted filtrate of the homogenised tumour produced an immediate type positive reaction when injected intradermally in the patient, but not in control subjects. The antibody could be passively transferred. The antigen was not present in the patient's own normal breast tissue. CURTIS et al. (1961) reported similar findings in a patient with dermatomyositis and bronchogenic carcinoma, to an extract of which she gave a positive immediate type reaction on intradermal injection. The passive transfer test was also positive. Polysaccharide tumour antigens are now thought to be commonly present in neoplastic tissue, and auto-antibodies may result from them (MAKARI, 1958), but there is as yet no evidence or to prove or disprove whether such antibodies are causally related to dermatomyositis.

Purpura.

The mechanisms by which the walls of capillary vessels are damaged so as to allow the diapedesis of red blood cells with resultant purpura are quite unknown. That it can be immunological is undisputed, examples being 'idiopathic' and drug-induced thrombocytopenia and thrombotic thrombocytopenic purpura. However, in most instances of so-called allergic purpura and allergic vasculitis no allergen is identified.

In one rare type of purpura with ecchymoses the cause appears to be acquired hypersensitivity to the red cell stroma, and it is termed auto-erythrocytic purpura (GARDNER and DIAMOND, 1955). Most cases follow physical trauma, an operation or haematoma formation at the onset. The patients are usually women, in whom purpuric papules, nodules and larger ecchymoses are found in the skin, especially on the legs,

thighs and hips. They appear spontaneously or after trauma and may be accompanied by fever and malaise. It is worse at the time of the menses. Some cases are associated with cerebral and internal haemorrhagic episodes. The lesions can be reproduced, by intradermal injection of the patient's whole blood or her erythrocytes (0.50 ml. of a 50 % suspension of cells) or the stromata from lysed red cells (0.1 ml.). Splenectomy, steroid therapy and desensitization are all ineffective but there is some tendency toward recovery with the passage of time, although cases have persisted for twenty years.

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TRATAMIENTO DEL ASMA BRONQUIAL

THE TREATMENT OF BRONCHIAL ASTHMA

TRAITEMENT DE L'ASTHME BRONCHIQUE

DIE BEHANDLUNG DES BRONCHIALASTHMA

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CONTROLLED EVALUATIONS OF EMULSION THERAPY IN RAGWEED HAY FEVER (*)

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During the 1960 ragweed season, the effects of an emulsion of 9 parts mineral oil, 1 part Arlacel A and 5 parts aqueous ragweed extract were evaluated in 38 previously untreated ragweed sensitive children and adults. All patients were given a series of "priming" doses of small amounts of aqueous ragweed extract in order to determine their tolerance. Twenty patients were then given a single injection of an emulsion containing from 193 to 3780 protein nitrogen units of extract depending on their reaction to priming doses. One moderately severe systemic reaction occurred. These patients were compared with 18 control patients of similar age, sex and threshold of skin test reactivity who received placebo emulsions containing no ragweed extract. Clinical evaluation of results was made in a double-blind fashion by seeing the patients weekly during the hay fever season and recording daily symptomatology and the amount of antihistamines and steroids used. Four of 18 placebo patients were considered 50 % or more improved by the evaluating physicians whereas 15 of 20 treated patients were considered so improved. No patient was completely free of symptoms.

In 1961, a larger study was performed, and out of 100 children and adults originally selected for treatment adequate data were obtained from 83 patients, 40 children and 43 adults. A 6.5:3.5:10 ratio of oil to Arlacel to extract was used and treated patients received a standard dose of 1000 p. n. u. of extract while control patients received indistinguishable placebo emulsions. Evaluations were performed in a double-blind fashion similar to those of the previous year. Twenty-six of 43 (60 %) of control patients were considered 50 % or more improved whereas 23 of 40 (58 %) treated patients were considered similarly improved. There was no difference in symptoms reported by the two groups of patients either when the pollen count was low or when it

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was high. Antihistamine consumption was the same in the two groups. Tanned human red cells coated with short ragweed extract were used in the passive hemagglutination test to determine antibody responses to the repository. There was no greater rise in titer among treated patients than among controls. The lack of clinical response and failure to demonstrate antibody response indicated that the dose used in 1961 was too low to be useful.

In 1962, a higher dose was used and treated patients received an initial dose of 500 p. n. u. mixed ragweed extract followed by a second dose of 2000 p. n. u. two weeks later. The method of emulsification was the same as in the previous year. Fifty-two adult untreated ragweed sensitive patients were selected who had a positive skin test to 0.1 p. n. u. or less of whole ragweed extract. The patients were paired as to age, sex, and duration of hay fever; one member of each pair received emulsions containing whole ragweed extract; the other received placebo emulsion. Ten of 26 treated patients had local reactions to both injections with swelling greater than 4 inches in diameter. One patient had hives. Placebo patients had no such reactions. Eleven of 19 treated patients had a 2 tube or greater rise in serum against passively sensitized tanned red cells. More careful methods of evaluating hay fever symptoms were adopted and patients were telephoned twice weekly and asked to rate the severity of sneezing; stuffy, runny nose; burning, itching eyes; and asthma. Patients also kept a daily record of severity of symptoms and antihistamines taken. Data from 18 pairs of patients were complete enough for evaluation. Comparison between the treated and untreated patients as to symptoms reported or medications taken failed to reveal any difference between the two groups. There was no evidence that protection was afforded to treated patients either on days when the pollen count was high or on days when it was low.

Because no benefit could be demonstrated in these two studies when previously untreated patients received emulsions for the first time, the effect of second and subsequent years of treatment was evaluated in 1963. Those patients who returned in 1963 for a second or third year of treatment were compared with previous placebo patients who now received specific treatment for the first time and a group of newly selected untreated patients who received placebos. Treated patients received 500 p. n. u. mixed ragweed extract emulsifield in Daroil 35 followed in 2 weeks by 2000 p. n. u., the second dose being given 2 to 3 weeks before the onset of the pollen season. No systemic reactions occurred, but the rate of severe local reaction was about the same as in 1962.

Results were evaluated as in 1962. Average daily symptom scores of specifically treated old patients were consistently less than scores of newly selected patients, even among the former placebo patients now being treated for the first time. Examination of prior records showed that the patients seen in previous years who agreed to return had reported lower symptoms than those who did not return, regardless of whether they had received placebos or specific therapy. Thus the apparent good results obtained with the second and subsequent years

of treatment appeared to be based on the refusal to return of those patients who had more severe hay fever, thereby leaving only the milder cases for retreatment. Patients being seen for the first time have not, of course, been subjected to this kind of selection, which serves to retain the milder cases.

By these methods, it has not been possible to demonstrate that emulsions of ragweed extract produce objective improvement of hay fever symptoms even in patients who have received them as long as 3 years. The number and severity of local reactions to the ragweed extract in these emulsions precludes further study with larger doses. These studies do demonstrate that the more severely affected patients may drop out of a course of prolonged therapy which affords little benefit, creating the false impression that the remaining mildly affected patients have improved.

THE OPSIPHYLACTIC TREATMENT OF RAGWEED POLLINOSIS (1963). THE USE OF EXTRACTS EMULSIFIED IN SILICONES

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Introduction.

The present report is one of a series of studies concerned with the treatment of pollinosis and also other sensitivities to inhalant substances and as well allergy to venoms of insects. The first published reports described the hyposensitizing effects of standard extracts emulsified in incomplete adjuvants of the liquid petrolatum type. Studies done with secondary adjuvant substances will be reported upon in the near future as will the effects of new types of extracts administered neither in emulsified nor unemulsified forms. Reviews of liquid petrolatum and of bee venom sensitivity are in process of preparation. This study is the first of a group of ten of which the chief subject is the use of silicones as vehicles for the emulsified pollen extract or the enhancement of adjuvant effects.

The Patients.

The present study concerns 1868 patients, male and female, adults and children, who had been afflicted with ragweed pollinosis for a minimal period of at least three consecutive years. Some patients suffered from hay fever and some from asthma and some from both disorders simultaneously or in succession or during different parts of the same season or during different pollen seasons and in a few instances for as long as sixty years.

An analysis of the effects of treatment of pollinosis by injections of extract demonstrated that the age and the sex of the patients were, neither of them, factors. The duration of the disorder made no difference in the results obtained. Although early investigators reported that it was easier to lessen the symptoms of allergic coryza as compared to those of pollen asthma we have discovered that all parts of the respiratory tract respond equally well.

The factors which appear to militate against complete relief of symptoms include multiple concurrent sensitivities, intercurrent or chronic infections of the respiratory tract, structural abnormalities incapable of being completely corrected, resentment toward being allergic and resistance toward being forced to take treatment by injections administered in accordance with traditional programs. The presence of allergic substances not identified but associated with given environments or seasons must not be ignored.

Analysis of the reports of others, as well as our own, demonstrated that when the diagnosis was accurate and complete then almost all of the patients could be made free of symptoms when they received enough of an extract of excellent quality and if treated opsiphylactically, the extract was administered in a good emulsion. Obversely, the patients who received a poor extract or an insufficient quantity of it or a large enough amount but poorly emulsified did not do as well as might be expected.

All of the patients filled in General Health Questionnaires and the other questionnaires which have previously been reported upon [1]. The possibility of food allergy or of drug sensitivity or of occupational or home environmental exposure to allergens was known before the initiation of injection treatment. Unless the referring physician had done a physical examination in the recent past the patient was checked for the presence of other disorders and when possible these were corrected. All of the supplementary examinations deemed necessary were done by consultants and when needed laboratory studies were requested, and in almost ever instance obtained.

Obesity and excess drinking or smoking were not permitted to go without attention, and although treatment was not in all instances completely successful, there were no patients in whom it could be said that the regimens imposed were complete failures if only because the patients who rejected the programs of control did not remain on as patients.

Previous treatment by means of injections had usually not been effective or if effective then not completely so or if the patient had been made well he had ceased taking his injections because of the tyranny of the year-long necessity for attendance at a physician's office. Because the extract differed from that which had previously been administered, it was necessary to treat all patients as though they had never before received any injections. It is true that there might have been some residual protection and that the new injections might then act as 'booster' treatments. It was also true that some of the patients recorded more severe symptoms with each successive year of exposure, and it was thought that the two factors would in some sense nullify each other as far as the over-all effects of treatment might be assessed.

The Skin Tests.

Although it is not believed that skin test reactions are, in every patient who suffers from clinical inhalant allergy, a proof of its existence,

the reactions are present in perhaps 90 per cent of the patients. So that this study would conform to those in the literature, patients who did not present typical skin test reactions were excluded from this particular report although a number were similarly treated. In those who had never before been studied or treated the preliminary tests were of the pressure puncture or scratch type. The others received intracutaneous tests with extracts decimally diluted as represented by 10, 100, 1 000 and 2 000 PNU/ml. If the larger skin test reaction to the more dilute extract is to be taken as an indication of greater sensitivity then it may be said that the reactions and the clinical histories were not often in agreement.

The patient whose symptoms began early in the pollen season and extended until the end of the season or beyond it and who was not entirely relieved by antihistaminic agents, or if his symptoms were alleviated it was not for long, was invariably discovered to be the more sensitive patient as regards local and general reactions subsequent to injection. There are times when the test reactions and the history agree, but when they are not in accord the clinical history was the more dependable guide to treatment.

The skin test responses were fairly evenly distributed among the 1 867 patients except for several in whom they could not be elicited except with solutions as strong a 4 000 PNU/ml. This was not thought of as important because some of these same patients, on other occasions that the first, were discovered to be reactive to extracts of greater dilution.

If the large skin test response signifies a state of reactivity to injected extract the tendency to react systemically may be minimized or abolished by the use of greater volumes of emulsion in which case the number of units injected need not be diminished if the emulsion is correctly prepared. It is of the utmost importance that the extracting fluid be buffered and balanced for all of the necessary anions and cations and for protein [2].

The Extract.

The extract of short ragweed pollen was prepared immediately before the treatment period by the method previously described [3] and standardized in Brown-Benotti protein nitrogen units [4]. It has been stated that extracts stronger than 40 000-50 000 PNU/ml. are difficult if not impossible to prepare and that the stronger extracts do not readily go into emulsion form. Neither in the present nor in previous studies have these problems been met.

The Vehicles For The Emulsion.

For the continous phase a pharmacologically inert silicone was used. Several of the psiloxanes are being studied and the decision as to the

one or several which will finally be used has not been made at the time of writing this report. Nor was the formulation of the silicone and the Isocreme (Croda, Ltd.) used as an emulsifying agent constant although the majority of the patients received a silicone: Isocreme: extract mixture in which the successive ingredients were present in the proportions of 4:5:9. The exact proportions of the agents and the extracts will be the subject of a separate report, now in press.

The Emulsion.

The emulsions were prepared on a Brown Emulsor in a manner previously described [3] and by means of the same 18 and 20 gauge emulsors, and for the same length of time. The energy content of the systems was the same and the range of the droplet size no different. When the emulsions were used for skin test purposes there were no positive reactions in patients who had by means of pressure puncture techniques demonstrated responses when solutions of equal of greater dilutions had similarly been used.

The Quantities Injected.

The same type of emulsion with perhaps slight differences in the proportions of the ingredients had been injected without the induction of systemic reactions in several thousand patients allergic to the pollens of the grasses and the trees and as well for the treatment of sensitivity to house dust, animal danders and insect venoms. It was thought wise to view each type of extract as though it represented a new problem in the hyposensitization of patients.

At the time their studies were done, approximately 300 patients received control injections of emulsified saline in a total volume of 0.5 ml intramuscularly administered only so that any local ill effects might be discovered in advance. In none were there any signs of any local or general effects.

The treatment period extended from early April until late August. The last two weeks of August represented the time of injection of patients who could not, for various legitimate reasons, keep their second appointments having received their first injections during June or July.

The first table needs little textual comment. It had been our intention to treat 200 patients with initial doses of 500 PNU/ml. The actual number was 222 in that seven patients received injections in accordance with the schedules listed in the second table. The total number of units administered is shown. The number of patients who reported having experienced symptoms will be discussed under the heading of *Results.*

We had intended to treat 1 000 patients with initial doses of 1 000, but the number so treated was 998 as taken from the first and second tables. It may be noted that the first patients of this group began with 1 000 PNU/ml, and, at intervals of two to four weeks, received 1 000,

TABLE I

TREATMENT OF RAGWEED POLLINOSIS 1963

Quantities of Extract (PNU) Administered According to Experimental Design (Chronological)

Number of Patients Treated	Numbers of Injections 1	2	3	4	Total Dose	Number of Patients Reporting Symptoms
80	500	1000	10000		11500	10
16	500	1000	2000	10000	13500	5
60	500	2000	10000		12500	6
11	500	5000	10000		15500	0
48	500	10000			10500	1
16	1000	1000	2000	10000	14000	1
131	1000	2000	2000	10000	15000	1
321	1000	2000	10000		13000	17
17	1000	2500	10000		13500	0
30	1000	5000	10000		16000	1
481	1000	10000			11000	24
110	2000				2000	0
42	2000	2000	10000		14000	5
106	2000	10000			12000	49
379	10000				10000	8
5	20000				20000	0
1853						128

2 000 and 10 000 PNU. As the study progressed the total volumes injected decreased so that the later injections of any series were usually administered as 0.2-0.4 ml.

The patients who planned to go abroad, or to spend their vacations in areas free of ragweed pollen, or whose exposure would be limited in quantity or duration were treated with single injections of 2 000 PNU-0.4 ml. Of these there were 110 and not one reported the experiencing of any discomfort. In almost all instances the reasons for the excellent results was the limited exposure. It should not be concluded that this quantity of extract is sufficient treatment for the average patient who is not so privileged.

With the approaching end of the treatment period, the number of patients treated with each quantity of extract diminished and only 151 (instead of 300) patients received 2 000 PNU followed by larger quantities, either 2 000, 3 000, 5 000 and then 10 000 PNU. Two patients were treated with 2 500 given initially and because they returned for second injections at a time when they were physiologically upset or were suffering from intercurrent infections, received second injections of 2 000 PNU before they were treated with a final dose of 10 000 PNU.

No reactions had been experienced by any patients and the next succeeding 379 patients, regardless of skin test responses received a single

injection of 10 000 PNU. The last five patients of the group studied received single injections of 20 000 PNU/0.4 ml. All had been treated successfully during the previous year. None reacted adversely.

The Results.

The effects of any agent used in the treatment of any clinical disorder for which there is no unit of measurement are difficult to evaluate.

The patient who is seasonally ill, but not always equally so, will take treatment in the hope that he will be much better than he otherwise would have been in the absense of the injections. There is no method of discovering whether the ensuing season would be one during which there would be minimal or no simptoms. There will always be a number of patients who are due to enjoy a spontaneous remission. These cannot, in advance, be distinguished from any other patients in whom the injections are the decisive factor in the freedom from symptoms.

There are patients whose records show that with each passing year they are affected earlier and more severely by their exposure to pollen. Any conclusion that they would continue with this patern is not entirely warranted, but is usually likely. One may safely infer that any patient who has in the past responded to treatment by injections and has been well and during the years when he has taken no injections has been ill will, if the pattern has proved itself to have been established by a number of such trials of treatment and no-treatment, will similarly respond in the future although, once again, this assumption is not capable of being proven.

It is certain that a number of patients will develop other seasonal sensitivities which may be concurrent or overlap with the season of the pollen for which the patient has received treatment. It may also be concluded that because allergic patients are multiply sensitive, some of the substances which are the causes of symptoms extra-seasonally will be the causes of symptoms which occur during the season. Unless such effects of exposures to substances other than the pollen for which the injections were administered are equal both during and before or after the season there is again no means of measuring whether one or both reasons for the discomfort existed in that the patient would have been less affected had there been no pollen in the air, or worse if he had not received the injections.

The placebo reactor who states that the injections have added to his discomfort and the placebo responder who reports that he is well if only because he took treatment and would have been equally well had the injections consisted of saline solution, is not a special problem because there will undoubtedly be equal numbers of these and their presence in any large series of patients may be expected no matter what the study may set out to discover.

There were only four patients of whom it might be stated with certainty that their symptoms were caused by exposure to the pollen of

ragweed. Their discomfort began when the pollen appeared in the air, and was the cause of equal discomfort during the same days and times when the control patients who had been referred during the season or who had reported too late for treatment or else had been treated in the past and had deferred any injections until symptoms had afflicted them. These four patients responded with the typical patterns of relief to the administration of antihistaminic agents which were required for all of the pollen season and for some days after the pollen was suppossedly

TABLE II

Quantities of Extract (PNU) Administered According To Skin Test Reaction or Patient's Condition

Number of Patients Treated	Numbers of Injections					Total Dose	Number of Patients Reporting Symptoms
	1	2	3	4	5		
1	500	1000	1000	10000		12500	0
1	500	1000	2000	2000	10000	15500	1
1	500	1000	10000	2000		31500	1
1	500	1000	5000	10000		16500	1
1	500	2500	10000			13000	1
1	500	3000	10000			13500	1
1	500	10000	20000			30500	0
1	1000	1000	3000	10000		15000	1
1	1000	2000	2000	10000		15000	1
1	1000	3000	10000			14000	1
2	2000	3000	10000			15000	0
1	2000	5000	10000			17000	1
2	2500	2000	10000			14500	0
15							9

no longer ambient. We are certain of three and almost certain of the fourth "failure" because, in his case, there is some doubt as to the influence of some secondary factors as exposure to house and garden plants to the pollens of which is was possible to elicit skin test reactions. In this patient such exposure could neither be eliminated nor avoided.

There were 133 other patients who returned to the office or were requested to return because of the discomfort they experienced and reported upon at the time. In each of these it was not difficult to establish to the patient's and to our satisfaction that there were secondary causes for the symptoms. The criteria used to establish such secondary causes were, in many instances, such that they would universally be acceptable.

There were the patients whose symptoms, according to their histories, occurred only when they were exposed to substances which extraseasonally also resulted in upper or lower respiratory tract signs and symptoms. These took place only at the time of exposure, although they might have persisted after the cessation of exposure but, at the most,

for a day or two. These same patients had antecedently been exposed to the pollen with no ill effects and subsequently often when exposed to increasing quantities of pollen as the pollen season advanced, again suffered from no inconvenience. It is possible that had these patients received greater quantities of pollen extract their thresholds might have been raised so that the secondary factors might have been the causes of less or perhaps of no difficulty. This conjectural statement represents a nonoperational condition which could not possibly be established satisfactorily because the amount of exposure of the secondary factor cannot be measured.

Some of these same 138 patients suffered from typical upper respiratory tract infections. Their symptoms lasted as long as did their "colds" which, in almost all instances, also affected other members of their families including those who had never shown any evidence of allergy. There were the usual number of patients who ate foods to which an allergy was known to exist. In some cases, the ingestion of the food substance was inadvertant, but in others especially when there was a craving for the interdicted food, it was eaten, as it were, on purpose. The symptoms lasted the usual two or three days, but occasionally longer than they might have extra-seasonally. There were no additional symptoms unless the patient once again indulged in the same experiment. In some patients of this type, treament with larger quantities of extract sometimes enables them to eat more of the food and often with no consequences, but how much treatment for how much of the suspected or known food sensitivity it is impossible to determine.

There were the patients who were exposed to the fumes of re-painted houses or offices and factories and those similarly exposed to the dusts of wrecking and construction projects as well as road building. It is interesting that they were once again free of symptoms when they could remove themselves from the environments of such exposures.

Ninety-nine of the 137 patients were also sensitive to the pollens of the grass some of which continued to pollinate intermittently through the months of August and part of the month of September. Such pollination would vary according to local conditions and vould not occur in patterns wich would be readily apparent. Of this same groups of patients, 84 reacted to tests with house dust extract, and there is the strong possibility that the occasional symptoms noticed when heating systems were first activated may have been evidence of clinical allergy to house dust. Two patients were allergic to plantain pollen. Thirty-five other patients of the group of 137 were allergic to house dust and not to the grass pollens, and a few others to both house dust and molds. In none of these were the patterns response those of the untreated ragweed pollen sensitive patients, and it is therefore concluded that they were not entirely, if at all, afflicted because they were not sufficiently protected against exposure to ragweed pollen.

It should be noted that among the group of patients treated with some consideration given to their skin test reactions and general health, there were more patients who reported symptoms (nine of fifteen) than

TABLE III

Distribution of Patients According to Symptoms Quantity of Extract Injected and Number of Injections

Total Quantity of Extract Injected	Number of Injections					Patien. Report. Sym.	Total Number of Patients Treated
	1	2	3	4	5		
10000	10000					8	(379)
10500	500	10000				1	(48)
11000	1000	10000				24	(481)
11500	500	1000				10	(80)
12000	2000	10000				49	(106)
12500	500	2000	10000			6	(60)
13000	500	2500	10000			1	(1)
13000	1000	2000	10000			17	(321)
13500	500	1000	2000	10000		5	(16)
13500	500	3000	10000			1	(1)
14000	1000	1000	2000	10000		1	(16)
14000	1000	3000	10000			1	(1)
14000	2000	2000	10000			5	(42)
15000	1000	1000	3000	10000		1	(1)
15000	1000	2000	2000	10000		1	(131)
15500	500	1000	2000	2000	10000	1	(1)
16000	1000	5000	10000			1	(30)
16500	500	1000	5000	10000		1	(1)
17000	2000	5000	10000			1	(1)
31500	500	1000		10000	20000	1	(1)
32000	2000	10000	20000			1	(1)

among those who were given injections with no regard to their skin test sensitivities (128 of 1 853).

The third table lists the patients in accordance with the total number of units injected, the number of injections and the number who received such injections and the total quantities injected and the number of each group who reported have experienced symptoms during the pollen season. Except for the 49 of 106 who were treated with injections of 2 000 followed by 10 000 PNU and reported symptoms would appear from scatter diagrams that there is no realationship between the dose and the well-being experienced. It would appear that doses of 10 000, 11 000, 13 000 and 15 000 PNU are most often followed by complete relief in the greater numbers of patients treated. There are exceptions to this apparent rule in that five of 42 patients treated with 14 000 PNU, and five of sixteen who received 13 500 PNU were uncomfortable. The symptoms of none of the patients were truly related to the dose administered granted that, on the basis of previous experience, it was more than enough. The symptoms were caused by other substances than the pollen of the ragweed and happened to occur during the pollen season.

If a patient receives a large enough quantity of a potent extract emulsified so that there are neither local nor systemic reactious and in

adequate time for him to develop whatever is meant by hyposensitivity, it may be expected that any symptoms which occur during the pollen season are caused by other inhalants, foods or non-specific factors. The silicones are useful as vehicles for emulsified extracts of pollens.

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THE PRESENT STATUS AND FUTURE OF EMULSIFIED ANTIGEN THERAPY *

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Various methods have been employed during the last thirty or more years to try to reduce the number of injections, to obtain better therapeutic results, and to increase the safety of desensitization in allergy. Water in oil emulsions have been used from almost the onset of these various studies because they provide an important physical method of slowing absorption. Early studies employing vegetable oils as a base were rapidly abandoned because of nodule formation and unpredictability of absorption. The studies of JULES FREUND using mineral oil emulsion with or without tubercle bacilli as an adjuvant stimulated Mary Loveless, and later others, to use this method, omitting the tubercle bacillus, in the treatment of human allergies. Various modifications of the proportions and ingredients have been tried but with no great difference in results. Many questions remain unanswered despite intensive work by many investigators over the past few years.

We know something of what happens when we inject an emulsion containing an allergen such as ragweed. A small but appreciable amount of the allergen is immediately available. There is continued absorption over the next 24 hours resulting in a fairly large release. After that, release is gradual and usually lasts for months but at a diminishing rate. Systemic reactions, while occurring in a smaller number of patients because fewer injections are given, do occur and are likely to be more prolonged. Local reactions may also be present and prolonged. A delayed reaction resulting in abscess formation may occur several months after the injection. We have evidence that this is more likely to occur in those patients who are not clinically sensitive to the allergen injected. In these previously nonallergic subjects an induced skin sensitivity, either immediate or delayed, may be produced by the injection of an emulsified antigen. This induced sensitivity may possibly be of some clinical significance but it provides us with an experimental tool to study the me-

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chanism of allergy in man. In other patients there is evidence that sensitivity to the allergen injected is increased after treatment.

Many of these objections could be disregarded if the results of treatment were substantially better than with the conventional aqueous treatment. In our hands, while individual patients may do better and others prefer this type of treatment because of convenience, most are better controlled by treatment with the aqueous extracts.

A comparison with the use of influenza vaccine in emulsion form provides a different result. Here the antigen used is less than that employed in aqueous treatment so the incidence of systemic side effects is essentially nil. The effectiveness in producing measurable antibodies to influenza virus is easily demonstrated. Antibodies in protective amounts may be found years after treatment.

What is to be the future emulsion therapy for allergy? New emulsifiers may be developed. New oils or other suspending agents offer some hope for further development and utilization of emulsions. Perhaps new principles of prolonging absorption of antigens might offer more hope providing antigenicity is not lost in the process. Actually there is evidence that if we make an emulsion too perfect immunization or desensitization does not occur, most likely because not enough of the antigen is released to stimulate the production of antibodies.

Among the questions still to be answered are:

1. What is the ultimate fate of the mineral oil and how much may be tolerated? The Food and Drug Administration and the Division of Biologics Standards of the U. S. Public Health Service are concerned about this and are encouraging a long term animal study in this field.

2. How stable is the emulsifier and the emulsion? This problem must be solved before these materials can be made commercially available.

3. Finally, the most important question remain unanswered. What do we really wish to accomplish in antibody production and what is the mechanism of relief desensitization in allergy?

THE USE OF EMULSIFIED EXTRACTS IN TREATMENT OF RESPIRATORY ALLERGIC DISEASE

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The specific treatment of respiratory allergic diseases, with either aqueous or emulsified extracts, usually is associated with satisfactory clinical results. It is important to determine as accurately as possible the clinical significance of skin test reactions. A good history is diagnostic of pollen or mold sensitivities. In the midwestern regions of the United States the pollen seasons, for the most part, are quite sharply demarcated. The onset and duration of symptoms not associated with pollen seasons emphasize the inconsistency with which mold symptoms are present throughout the spring, summer and fall. Local conditions —a farming area, the proximity of cereal mills and grain elevators— do play important roles affecting mold patients in the exposure or degree of distress and in the anticipated measure of relief to be provided by any method of therapy. The etiologic diagnosis is confirmed by positive skin test reactions with their clinical significance revealed by the history.

Emulsified extracts for treatment are prepared in the following manner: the oil and emulsifying agent * is a single unit designated as Drakeol-Arlacel AVR 35 supplied in 4 ml amounts. To this is added an equal volume of the aqueous extract, in a PNU/ml strength to bring the end product (the emulsion) to the desired dosage level. Diluting fluid, as buffered saline, is added so that the total volume of the preparation is 8 ml. The aqueous-oil mixture then is emulsified in mechanical equipment preferred by the physician.

Prior to the use of the emulsion in treatment, it must pass a critical survey. Low power microscopic examination reveals a uniformity of small droplets when the emulsion is compressed between a slide and a cover slip. As a positive scratch test with the un-emulsified extract is considered important, so a negative response on scratch test with the emulsified extract is significant of the small amount of free, aqueous extract in the emulsified material. The "feel" is based on the manner in which the emulsion is worked from syringe to syringe through the emulsifying needle or in the emulsifying apparatus.

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I know of no reliable means by which a patient's dosage can be estimated, and there is no reliable guide (other than experience) to determine the amount of extract, either emulsified or aqueous, that a patient will require for adequate treatment. The size of the skin test reaction and/or the level of conjunctival reactivity are not dependable nor accurately suggestive. The patient with mold sensitivities does not have conjunctival symptoms to the degree of the pollen sensitive patient. Therefore it does little good in mold-sensitive patients to record titration based on conjunctival responses. Some investigators have proposed a "provocative skin test" wherein a systemic reaction is produced by titration with strengths of extract intentionally given to exceed the patient's level of tolerance. The treatment dosage is maintained just below or at this responsive, indicated level. The value of such provocation is open to question.

The dosage schedule for every patient must be individualized regardless of the method of treatment or the extract employed for relief of symptoms. For the previously untreated pollen sensitive patient, a priming dose of emulsified extract is given six to eight weeks prior to the expected onset of the season. This priming dose ranges from 1 000 to 2 500 PNU given in a volume of 0.25 ml. About two to four weeks after this primer, the treatment dose is given as 5 000 to 10,000 PNU in 0.25 ml volume. Booster doses of one-fourth the treatment dose may be administered during the pollen season if necessary and indicated by the presence of clinical symptoms.

Based on a few unpleasant experiences resulting from what now would be considered by me to be extremely large doses, my treatment schedule or plan for emulsified mold extracts has gradually but consistently been reduced to a low, but a satisfactory amount. Alternaria is by far the most prevalent and most important mold causing respiratory allergy in my part of the country. If the history of onset, the duration of symptoms and the degree of exposure suggest that the patient is primarily (or only?) sensitive to alternaria, 200 to 250 PNU of emulsion are the treatment dose for that patient. If the patient is considered to be sensitive to four or five members of the dematiacious group—or to other significant molds—the "mold-mixture" dose would remain at 200-250 PNU for each mold, with a total of 1 000 PNU as the maximum for any single injection. This dose, for either the single or the multiple emulsion, in 1963 was given in 0.5 ml volume. This year, 1964, the volume has been further reduced to 0.25 ml of emulsified extract.

These treatment doses are given subcutaneously, using a disposable tuberculin syringe and a disposable 25 gauge needle, 5/8 inch in length. When it is determined that a patient requires a "booster" dose because of symptoms in season, one-fourth to one-half of the treatment dose is injected intra-muscularly. This has been a satisfactory dosage routine associated with little, if any, local irritation or swelling, and conducive to an excellent clinical result.

There have been few reactions—less and less frequent with experience. Most of the reactions associated with pollen or mold emulsified

extracts have been redness or mild swelling at the injection site. The middle third of the outer aspect of the arm is the usual location for the administration of the emulsion. Emulsified mold extracts have produced only two severe reactions characterized by respiratory symptoms. The onset, in each instance, was about one to two hours after injection and was, I am sure, caused by excessive PNU doses—a level that has not been used in recent years. The absence of local or systemic reactions is thought to be due to the use of sterile, disposable equipment, the preparation of a better emulsion, and a dosage more suitable to the patient's degree of tolerance.

Cysts or nodules have been remarkable in their rarity during these years of reduced, more suitable ranges of dosage. A sterile abscess has been associated with the use of emulsified extracts in each of seven patients: one dust extract 3 500 PNU/ml; two mold extracts 1 000 PNU/ml; two grass pollen extracts 5 000 PNU/ml and two ragweed extracts 7 500 PNU/ml. The diagnosis was made four to six weeks after injection in three patients, three months after injection in two individuals, and four to six months in the other two patients. Aspiration of each sterile abscess area was accomplished with a syringe and an 18 gauge needle. The volume of material removed ranged from 20 ml to 85 ml. The contents of each cyst were light tan in color, moderately thick in consistency and sterile on culture. After each aspiration, injectable steroid was placed into the aspirated area. There was complete regression and healing with only a resultant dimple within three to four weeks after the original aspiration. Surgical incision or drainage was not necessary in any of these complications. Five of these seven patients have received emulsified extracts without complications of any type in subsequent seasons.

The age of the patient has no influence upon the decision to use emulsified extracts of mold, pollen or other material. The ages of the patient in these years of treatment ranged from two years to seventy-four years. The majority of patients who receive specific treatment to relieve the symptoms of respiratory allergic diseases have been well-satisfied and comfortable. In my opinion, the advantages and benefits found with the use of emulsified extracts are to be preferred to other measures and methods of treatment.

THE EFFECT OF SPECIFIC HYPOSENSITIZATION ON HUMAN BLOOD HISTAMINE RELEASE

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The *in vitro* liberation of histamine by the action of antigen in the blood of allergic individuals has become an increasingly informative phenomenon, for it is now used to investigate a number of aspects of atopic reactions. In differing experimental models in which histamine is released by the effect of antigen-antibody interaction, there are always two requisite parts: the first part is the intact, living, hypersensitive cell and the second part the specific allergen. In the particular example to be discussed here the cells are those obtained from an atopic individual and the specific antigen is the allergen involved in producing the patient's symptoms. The leucocytes from an individual may react to a number of antigens. Indeed, release by various allergens may occur in aliquots of an individual's blood so that the release of histamine or the absence of release may be demonstrated and may be of some diagnostic significance.

The histamine release phenomenon is peculiar to atopic reactions. The amount of histamine released correlates well with the gradation of the immediately reacting, wheal and flare type of skin test reactivity. With other varieties of antigen-antibody reaction, for example with tuberculin sensitive leucocytes and tuberculin as the antigen, histamine release is not demonstrable except at high concentrations of antigen, when the effect is nonspecific. Using the leucocytes themselves as the antigen in the presence of anti-leucocytic serum, no histamine will be released, although a concurrent antigen-antibody reaction of the atopic variety will release histamine in the usual amounts.

In performing release tests there are, of course, variations in technique. The leucocytes may remain in the blood sample with which they were withdrawn from the individual by venipuncture or they may be separated by centrifugation, washed in saline or an isotonic nutritive mixture and resuspended in the aqueous medium. A known amount of allergen is then added to the whole blood or the fluid in which the leucocytes are suspended, and the mixture is incubated at thirty-seven degrees centigrade. After a predetermined incubation period, usually fifteen or twenty minutes, the mixture is centrifuged to separate the

cells from the liquid. The liquid is withdrawn and its histamine concentration is determined. Comparison of the histamine concentration of the plasma or other liquid containing the allergen with the histamine concentration of the liquid obtained from an aliquot containing no allergen will reveal the amount of histamine released by the specific antigen-antibody reaction.

The histamine release procedure has already been found useful in many ways. It serves to indicate clinical sensitivity sometimes even when skin reactivity to the involved allergen can not be demonstrated. Such conditions as pH, temperature, tonicity and other factors attending this *in vitro* reaction may be altered as desired. Pharmacologic agents, metabolites and various extraneous substances can be added easily to observe their effect on the amount of histamine released. The influence of these conditions and agents may be appraised and thus further information concerning the fundamental nature of atopic reactions may be obtained.

A technique devised by Osler has been employed to quantitate allergenic potency. In this instance the leucocytes are washed to avoid the influence of humoral factors. The method is apparently quite sensitive, and may find use in standardizing, in a sense, allergenic activity as well as quantitating antigenicity.

Following hyposensitizing injections, altered reactivity of the blood plasma to antigen can be demonstrated by means of histamine release. This is, of course, the aspect of histamine release with which we are chiefly concerned at present. Van Arsdel and Middleton, studying histamine release during the course of hyposensitization of grass or ragweed sensitive individuals, compared release in the blood at the start of treatment with histamine release in the blood of the same patients after hyposensitization had been carried out. Using varying concentrations of antigen in the blood they were able to demonstrate that maximum release generally required greater concentrations of antigen after the course of hyposensitization. Further, they were able to correlate in a few individuals an excellent response to treatment with a great diminution in blood histamine release following treatment. In further studies by Van Arsdel the humoral nature of the post treatment factor was demonstrated by allowing the reaction to take place in Tyrode's as well as in plasma. Using thrice washed leucocytes and an incubation period of one hour with a final concentration of 0.01 micrograms of nitrogen per milliliter, the presence of plasma did not alter the amount of histamine released in eight untreated grass sensitive individuals. In treated individuals, with the cells suspended in Tyrode's, antigenic histamine release was quite similar to that obtained in untreated patients. In contrast, almost no release occurred in the presence of post treatment plasma unless a higher antigen concentration was used. The required concentration was found to be 0.1 mcg. of antigen nitrogen per ml, a tenfold increase over the antigen concentration required to produce satisfactory release in the blood of untreated patients or with the cells from treated individuals suspended in Tyrode's instead of in plasma.

Since histamine is one of the mediators and perhaps the chief mediator responsible for atopic symptoms and since a clear cut *in vitro* demonstration of the effect of specific therapy on production of the sympton-producing substance, histamine, has now been achieved, further experience with these methods may indicate that correlation of the clinical results of treatment and changes in *in vitro* blood histamine release may be closer than correlation of treatment results with changes shown by other immunologic techniques.

ASTHMA AND ITS TREATMENT

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When doctors from different countries describe the methods that they recommend in the management of asthma, it is likely that there will be considerable disagreement. An expert in one particular form of treatment may stress the advantages of this treatment over all others and he becomes a completely biased observer. The problem of observer variations in symptoms or diagnosis both by the patient and doctor may be very great. There is no doubt that patients with very little encouragement will describe symptomatic changes which cannot be supported by objective evidence. Asthmatic patients of any age group usually learn to live within the limits of their disability. They say they are much better, housework is possible or no time is lost off work. Yet objective testing using simple respiratory function tests may show no change for better or worse.

Everyone is agreed that a good history obtained from the patient is well worth the time and trouble. Yet it is as well to remember that both the giving and taking of the history may be extremely biased. A married woman will often relate the onset of asthma to marriage or particularly to pregnancy. This is probably due to the fact that there is a great tendency for people to atrribute onset of disease to some specific episode. Clinical histories suggest that persistent breathlessness often begins with an acute bronchial or pulmonary infection. The pathologist reports a causal relationship between bronchial inflammation and emphysema (REID, 1954). It is assumed that emphysema is responsible for the breathlessness, but severe airway obstruction may be asymptomatic. It may be that this obstruction increases the liability to infection (especially in a cigarette smoker). This infection sets up increased bronchial secretion which causes an increase of airway obstruction resulting in symptoms (FLETCHER, 1963). This sequence of events is exactly opposite to that usually accepted by current clinical and pathological hypotheses. These remarks about symptomatic changes that the patients describe to their doctors, are made with the idea that in future we can be more objective in our observations. We live in an age when machines can make measurements and we must learn to use these machines and at the same time learn their limitations. The wheezy pa-

tient is a demanding patient. He wants to know a lot about himself. He wants to know how much help can be given to him and he particularly wants to know what is his prognosis. He may be frightened by the acute attacks of asthma. He will want to know whether he may die in one of his attacks, or whether his heart is liable to be affected. Most patients however are optimistic and will want to know whether they can be cured. The English word "cure" may be difficult to translate into French because in France patients annually take "the cure". I do not think many doctors can claim that they are able to relieve all the symptoms of asthma in their patients but many doctors confidently tell parents that their children will grow out of asthma. The doctors are often correct in their prognosis which is made on quite unscientific grounds. It is a very good rule to remember that asthma due to one cause is rare (WILLIAMS, 1959). Asthma is much more often due to a combination of allergy, infection emotions and causes unknown, although in any one patient there is often, depending on his age, a predisposing cause. The patient may have preconceived wrong ideas as to the cause of his asthma. The doctor, especially if he be a psychiatrist or an allergist, may also be biased in his ideas. The all important history under these conditions may be almost valueless.

Definitions of asthma.

Confusion may arise from the biased opinions of the patient or the doctor about asthma, but more confusion still arises because we are largely unable to agree on the meaning of the terms used in bronchopulmonary disease. It is no use saying that bronchial asthma is an allergic conditions. This conveys very little information and the word allergic would have to be clearly defined.

RACKEMANN (1947) tried to sort out some of the different types of asthma when he coined the words "intrinsic" and "extrinsic". Intrinsic does not mean that the cause is not allergic, rather that the cause has not been found. When Rackemann followed up his cases long enough he found (RACKEMANN and EDWARDS, 1958) that many extrinsic allergic asthmatics became intrinsic in type. It thus comes about that if we wish to refer to asthma as due to a specific cause we tend to use the term "allergic asthma". Chronic bronchitis can be defined as "a disease characterized by a persistent cough and expectoration due to excessive mucus secretion in the bronchial mucosa". As this progresses and probably because emphysema develops, the patient starts a paroxysmal dyspnoea due to variable airway obstruction. He has bronchial asthma or it may be called infectious bronchial asthma. These terms are describing a symptom of the disease and referring to a morbid anatomical state. Unfortunately the pathology but not the symptoms of extrinsic asthma and infectious bronchial asthma may be quite different. It has been pointed out (GOUGH, 1961) that destructive enphysema does not occur in asthmatics. Yet some pathologists have described destructive emphysema as part of the typical histological picture of the asthmatic

lung. Presumably they are describing a different clinical condition. Biopsy specimens in asthmatics have shown an eosinophilic infiltration of the submucosal coat which is not present in bronchitis (GLYNN and MICHAELS, 1960). In summary, I would agree with SCADDING (1963) when he defines asthma in the following way: "Asthma is a disease characteterized by variable dyspnoea due to widespread narrowing of peripheral airways in the lung and varying in severity over short periods of time either spontaneously or as a result of treatment".

I believe that we may well have disagreements in our discussions on asthma, but these are largely due to the difficulties we encounter when trying to understand each other's terms. Unfortunately it is not easy to arrive at agreed definitions for allergy or asthma, but if we attempt to define our medical terms then I believe we will reach a measure of agreement.

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TREATMENT OF BRONCHIAL ASTHMA IN CHILDREN

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Early and proper treatment of bronchial asthma in children is of utmost importance, not only for the patients themselves. The allergy problem as such is also involved. One need only to consider the established fact that the adult's asthma has to a greater part had its origin in childhood.

The prophylactic treatment of asthma really should be started before the child is born. When one deals with an allergic family it is wise to observe the foods eaten by the expectant mother and especially her food cravings. These foods can be the first allergens to affect the child. Restlessness, vomiting, diarrhea and malnutrition may indicate the first signs of an allergy-to-be and lead to infantile eczema or asthma. A proper attention to these signs can help to prevent bronchial asthma which, as we know, can in about 50 % develop in connection with infantile eczema. Not only the food and drugs used by the nursing mother, but also the nutrition and drugs given to cows should be taken into consideration.

When dealing with allergic patients, both children and adults, a thorough history must be taken and be followed by a minute medical examination, necessary x-rays, laboratory studies, skin and provocation tests. Sometimes even psychological tests are called for. Adequate treatment depends entirely on these examinations. Specific treatment such as hyoposensitization will be discussed in an another connection. This paper deals with the so-called *general* (unspecific) *treatment*. The purpose of this treatment is to strengthen the allergic child's response to the disturbances caused by the main allergens, infections, other physical and psychic stress in order to make possible the change from an allergic state to a normal one or at least from a manifest to a latent, symptom free allergy. This considers simple causes such as anemias, poor nutritional, resting and sleeping habits. Correction of these can be rather easy by help of simple drugs, vitamins, roborantias, exercise and electroaerosol treatment. But the purpose of the general treatment is also to correct hormonal, metabolic and psychic disturbances found in the allergic child. Many of these demand hospitalization and special studies.

Patients with poor carbohydrate metabolism often seen in connection with insufficient function of the suprarenal cortex are given low carbohydrate diets. In these cases the Finnish sauna has been useful because it increases the function of the suprarenal cortex. Specific treatment is combined with unspecific such as sulphur in oil, histamine-azoprotein and histaglobin injections. Chronic infections in different parts of the body, mainly in the upper and lower respiratory tract, are—at least in the Northern countries—a common cause of bronchial asthma also in children. Infections of the nasal sinuses, adenoids, tonsils and teeth are often seen. Anti-infectious treatment is of great importance. It includes focal sanitation, combined with the use of antibiotics, stock- and autovaccines and gammaglobulin.

These angles of general treatment will be more closely discussed. Slides from the Hospital for Allergic Diseases in Helsinki, Finland show forms and results of treatment.

THE PATHOLOGY OF CORTICOSTEROID-TREATED ASTHMA

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Patients requiring large daily doses of corticosteroids to control asthma frequently develop the signs and symptoms of Cushing's Disease. Under such circumstances one is not certain that the benefits from treatment outweigh the side effects. Scant attention has been directed at the pathologic anatomy of Cushing's Disease except for the changes in the pituitary body and the adrenal glands, and there are no reported studies on patients with corticosteroid-treated asthma who died and came to autopsy.

The studies of cases of asthma coming to autopsy have indicated that when symptoms of asthma begin after the age of 40 years and are continous the average life span is 3 to 4 years. This life span might be lengthened by treatment that is the result of advances in the health sciences.

Five cases of continous asthma with onset after 40 years of age have been treated with large daily doses of corticosteroids for an average of 4.4 years. These cases lived an average of 6.9 years after the onset of symptoms. At death they came to autopsy and the pathologic tissue alterations are as follows: 1) decrease in the inflammatory reaction, the mucous gland activity, the basement membrane prominence, and the smooth muscle hypertrophy of the bronchial and bronchialar walls, 2) atrophy of the zona fasciculata of the adrenal cortices, 3) cytoplasmic hyalinization and vacuolation of the pituitary basophiles and hyperplasia of the pituitary acidophiles, 4) atrophy of the lymphoid tissue of the spleen, 5) hypertrophy of the myocardial fibers, and 6) slight and infrequent osteoporosis.

Long-term, high-dose corticosteroid therapy for asthma that begins after 40 years of age and is continous, is justified. The favorable anti-inflammatory effect of corticosteroids on the bronchi and bronchioles probably accounts for an extended longevity. The other structural tissue changes related to corticosteroids seem to be lesser evils than the consequences of the disease.

ROLE OF EMOTIONS IN CHILDREN WITH BRONCHIAL ASTHMA

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Asthma in children is certainly broadly recognized as being immunologic in origin. In spite of the immunological origin of asthma, the treatment of the total child must include the feelings of the child. The feelings of the child are inextricably bound with the attitude of the parents towards the child's disease and the child himself. In addition, we have also studied the attitude of the siblings towards the asthmatic child himself.

Our research in the past ten years has disclosed that our original classification of asthmatic children into two groups, those responding, and those not responding to specific hyposensitization therapy, is still valid. Our concern is with the children of the latter group, popularly referred to as refractory or intractable asthma, which implies failure to respond to traditional allergy treatment adequately administered for one or more years. It is conservatively estimated that there are at least 200,000 intractably asthmatic children in the United States requiring special tecnique of management. Psychotherapeutic processes are either too slow or invariably fail to take care of the emergency as long as the children continue to live in their own homes.

The kind of therapy to be predictably effective has resolved into the in-patient residential treatment today administered in 14 convalescent homes in the United States and Canada. The general nature of the rehabilitation programs in these institutions are fundamentally alike with important differences in detail. The total number of available beds is less than 600.

It must be borne in mind that hospitalization of the child includes not only separation from possible physical allergens, but also from the source of treating emotional conflict within the home of the asthmatic child. This temporary separation or "parentectomy" in our opinion is a very important therapeutic vector. Irrespective of the nature of the mechanism separation of the intractably asthmatic child for a suitable period of time permits the child to become tractable to hyposensitization treatment if necessary after his return to his own home.

THE CONTROL OF CHRONIC ASTHMA

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Since the last meeting of the International Congress in New York in 1961, there have been no significant advances in therapy of chronic asthma. In fact, during the past three decades, despite intensive investigations in many countries, there have been only two major notable advances: the introduction of antibacterial agents—the sulfa compounds and the antibiotics, and the introduction of adrenocorticosteroids.

Among allergists there is general agreement concerning the symptomatic control of the asthmatic state, the minutae of which need not be discussed; what differences in opinion may exist depend, usually, upon the clinical acumen, experience, and judgment of the attending physician. Optimal control of the asthmatic state in the final analysis will depend upon due appreciation of the multiple etiological factors involved in each patient, which cause or aggravate the disease, and are deduced from the information obtained from a detailed history, physical examination, and laboratory data, plus an evaluation of the skin tests.

Although skin tests have been employed in the diagnosis of allergic diseases for a half century, brief comment on interpretation with regard to identification of the specific causal antigen or antigens seems justified, as this question has important diagnostic, therapeutic and socio-economic implications. When positive, they serve only to place the patient in the atopic group; they do not specify etiology. The intensity of the reactions indicates with considerable latitude the initial dosage of antigen employed in desensitization. On the other hand, inhalent allergic asthma occurs in the absence of positive skin tests and even the absence of positive eye and inhalent tests. Despite the differences of opinion concerning etiology in the skin-negative group, to ignore this fact is to deprive the individual patient of the possible benefits of desensitization. On the other hand, in not a few patients, skin tests may be positive yet the history does not suggest inhalent sensitivity. A trial course of desensitization nevertheless is justified on the basis that the degree of sensitivity may be minimal yet accumulative.

Symptomatic therapy depends first on establishing good patient rapport, and the giving of ample reassurance, and the judicious use of

bronchodilator drugs, expectorants, antibacterial agents, and desensitization. Adequate control is attained in the majority of patients. In the final analysis, desensitization is the only known form of treatment which can produce permanent improvement. If refractory to the above therapy, more elaborate environmental control, such as airconditioning of bedroom or home, of removal to pollen-free areas may be advised, providing economic and other factors permit. Finally, if the disease fails to respond to the above measures, consideration of adrenocorticosteroids inevitably arises. In the absence of specific contra-indications, the known hazards of administering such therapy for periods of several weeks is extremely remote; in the case of perennial inhalent asthma, when continuous steroid administration for long periods may be necessary, one must balance the potential hazards against the hoped-for benefits and, again, this question depends upon clinical judgment.

Whereas an antigen-antibody relationship is accepted in the pathogenesis of inhalent allergic asthma, definite proof of such a mechanism in "infective asthma" is lacking. Many investigators support the concept that "infective asthma" represents hypersensitivity to bacterial fractions and the natural history tends to support this viewpoint: attacks of wheezing associated with acute respiratory infections may occur at odd intervals for years before the chronic phase becomes established; during this conditioning phase, sputum and blood eosinophilia develop and antibacterial agents and steroids suppress the process. The "infective form" of asthma also ensues when allergic inhalant asthma, either seasonal or perennial, becomes complicated by respiratory infection which aggravates the original state. At this time the clinical picture may simulate "pure infective asthma" and only by history can the inhalent phase be differentiated from the latter "infective" phase. Bronchitis and/or sinusitis, with or without nasal and sinus polyps, characterize both inhalent and infective asthma but are definitely more frequent in the latter. Eosinophilia of the sputum or of the peripheral blood, frequently of marked degree, characterizes the infective form of asthma, contrary to the inhalent allergic type. "Infective asthma" as defined here appears to be synonimous with intrinsic asthma (RACKEMANN) and eosinophilic bronchitis. These three conditions appear to be induced by respiratory infections and may be complicated by the formation of polyps, pulmonary infiltrates and, in rare instances, pericarditis, and Loffler's syndrome, representing the more acute variety of eosinophilic infiltration could, with some logic, be included in this category. Recently, our English confreres have reported a high incidence of aspergillis fumigatus in the sputum from asthmatic patients presenting high eosinophilia. Such fiindings have been uncommon in the United States, and more observations on this particular question are certainly indicated; likewise, monilia, heretofore rarely considered of etiologic significance, has recently been reported as a not infrequent cause of childhood asthma and, therefore, of more significance than a casual inhabitant of the respiratory tract. More study on this particular question is obviously needed.

When the physician is confronted with a patient in whom infection seems to be the primary role in precipitating the asthmatic state, skin tests may be positive to inhalent antigens but the etiologic significance can only be proved by a trial course of desensitization. On the other hand, patients with infective asthma may also develop specific and definite inhalent hypersensitivity, and this possibility too should always be anticipated.

Simple symptomatic therapy may be sufficient to control infective asthma when seen in its earliest stage. One may observe mild or even severe isolated transient attacks of asthma associated with apparent infection when eosinophilia of the sputum and/or blood may be noted. However, if the condition becomes chronic, more definitive therapy should be directed toward the control or elimination of infection by administration of antibiotics and, if sinusitis and polyps are present, by operative procedures including exenteration of sinuses and polypectomy. If such procedures are instituted before an irreversible phase has been reached, the asthmatic state may be favorably modified; if not, the process gradually reaches an intractable form. In such patients, continuous adrenocorticosteroid therapy, supplemented by antibotics for exacerbations, due to infection, is the only treatment whereby effective control of chronic asthma is attained.

Asthma, either with or without nasal polyps, in whom there is no evidente of infection or hypersensitivity to inhalent antigens, foods and drugs, but in which aspirin sensitivity frequently occurs is widely recognized. The etiology is obscure. Many patients in the past have been subjected to multiple polypectomies. Again, the only decisive therapy is continuous administration of adrenocorticosteroids, and such patients have been treated for years pending the discovery of newer diagnostic methods, which one hopes will reveal specific etiology and hence, more specific therapy. In fact, the incidence of operative procedures on sinuses, and polyps, have decreased during the antibiotic-steroid era.

Bacterial vaccines, either autogenous or stock, have long been used in the therapy of infective asthma. A recent controlled experiment suggests that such therapy is helpful but not curative. Much difference of opinion is held on this question and more comprehensive studies are needed to clarify the question.

Drug sensitivity of low-grade intensity sometimes is uncovered as an aggravating cause of asthma. Acute aspirin sensitivity in the nasal polyp-asthma group is well-known; it also occurs in the inhalent sensitivity patients but is less frequent.

Food allergy as the major cause of chronic asthma, has been and is still enthusiastically supported by some allergists, while contrary opinions, equally enthusiastic, are held by others. There is no doubt that food hypersensitivity is of significance in the cause of chronic asthma but the argument will not be resolved until a comprehensive, adequately-controlled study has been carried out to establish beyond doubt the precise role that food allergy plays in chronic asthma.

Some of our surgical colleagues have in recent years enthusiastically sponsored carotid body excision in chronic intractable asthma. Discussion with physiologists and neurophysiologists reveal little physiologic basis for bilateral, let alone unilateral, glomectomy. An adequately controlled investigation, utilizing sham operations, has never been carried out. The operation is not without some danger and several post-operative deaths have occurred. At present, therefore, one must regard glomectomy with great caution and considerable skepticism.

The treatment of status asthmaticus is well-recognized and the details need not be discussed. Emphasis should be placed more on prompt recognition and treatment of increasing signs and symptoms of severe asthma before actual status develops. Close observation of the patient should alert the physician to this possibility. Acute infections, profound emotional stress, excessive physical fatigue, exposure to an overwhelming amount of antigen all favor the development of severe asthma which should be vigorously treated to prevent the critical state. There is no question that steroid withdrawal by the patient or even the physician has been responsible for the development of severe, and even fatal, asthma.

Finally, the recognition and appreciation of stress: emotional disturbances, fatigue, pressure aspects of modern life and business, and undue physical exertion, and others cannot be overemphasized. Inability to adequately control these factors may negate all other forms of therapy.

SYMPOSIUM

ENFERMEDADES POR AUTOSENSIBILIZACION

DISEASES BY AUTOSENSIBILIZATION

MALADIES PAR AUTOSENSIBILISATION

AUTOSENSIBILISIERUNGKRANKHEITEN

E. Witebsky

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THE MEANING OF AUTOSENSITIZATION IN HUMAN PATHOLOGY

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Ever since the classic investigations of Ehrlich and Morgenroth, the failure of the living body to form autoantibodies which could be harmful to itself has been recognized as a biological principle, originally referred to as *horror autotoxicus*.

Burnet and Fenner attributed to the living body the capacity of distinguishing between "self" and "not self", termed the self-recognition mechanism. However, certain exceptions to this rule have been known for a long time. For instance, paroxysmal hemoglobinuria, a rare blood disease, is caused by an antibody-like factor in the patient's serum which unites with the patient's own blood cells when the temperature is sufficiently lowered, and then when normal body temperature is restored, lysis of the sensitized cells occurs.

The concept of autosensitization as a pathological principle in human disease has been amply supported by experimental findings. Three major experimental autoimmune diseases can be produced in animals—allergic encephalomyelitis, chronic thyroiditis, and aspermatogenesis. These experimentally produced diseases are extremely specific inasmuch as they can be elicited only when the corresponding tissue is used for immunization, i. e., brain tissue, thyroid tissue, and spermatozoa respectively, usually incorporated into complete Freund adjuvants. The nature of the mechanism of tissue destruction caused by autosensitization is still unresolved. While circulating antibodies cannot be excluded as yet from being involved in the tissue destruction, it is more likely that immunologically-competent cells of the lymphoid system play a major role.

The question, then, whether the human diseases corresponding to these three animal diseases can also be explained on the basis of autosensitization has been the topic of recent investigations. The most thoroughly investigated human disease is probably chronic thyroiditis, sometimes called Hashimoto's disease. While the demonstration of circulating antibodies is very suggestive of the autoimmune nature of this disease, the equivalent in the human disease of the role of the complete Freund adjuvant in the experimental disease has not been determined

as yet with certainty. Perhaps a previous virus infection of the thyroid gland constitutes the trigger mechanism, or a genetically controlled abnormality of the antibody-forming system might come into play. Abnormalities of the immunological apparatus might also be the basic mechanism resulting in such autoimmune diseases as lupus erythematosus and acquired hemolytic anemia.

The concept of autosensitization as the cause of pathological changes, once considered unrealistic, is now finding wider and wider acceptance.

AUTO-IMMUNIZATION AND THE LESIONS OF AUTOIMMUNITY

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The subject of the present paper was discussed in some detail in a review presented two years ago [1]. There have been several new discoveries of significance in the intervening period. These are included in the following brief discussion.

Mechanisms of Auto-Immunization.

1. Tissues separated by physiological barriers from the lymphatic apparatus may be regarded as foreign and readily induce auto-immunization in otherwise normal experimental animals and in man. The lens, uvea, central and peripheral nervous system myelin, thyroid, testis, and adrenal, as well as the skin, kidney, and gastric mucosa have been used in well documented animal experiments. In each case injection of tissue, with or without certain adjuvant materials, gives rise to autoimmunization (both formation of humoral antibody and sensitization of the cellular type) and to formation of lesions in the corresponding tissue of the immunized animal. Comparable human diseases induced by surgical or accidental trauma or, indeed, by injection of tissue suspensions are well-known. Among these are such lesions as "phakoanaphylactic" endophtalmitis, sympathetic ophthalmia, and the encephalomyelitis which follows rabies vaccination (*i. e.* injection of rabbit spinal cord).

2. Tissue components may be sufficiently altered by infection, irradiation, or burn, by treatment with denaturing agents or proteolytic enzymes, or even by conjugation with haptens or foreign proteins, that they are regarded as foreign by the lymphatic apparatus. Thus autoantibody, and in a number of instances cellular (delayed) sensitivity, have been produced against constituents of the liver, kidney, and spleen, against skin and gastrointestinal mucosa, and against both cellular and soluble elements of the blood. Again the same process may be observed both in animal experiments and in man. A well-recognized example is paroxysmal cold hemoglobinuria, a sequela to syphilitic infection in man.

3. Injection of heterologous tissue, which contains constituents cross-reactive with similar components of the host's tissue, has been

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found to induce the formation of antibody capable of reacting both with the immunizing antigen and with the host's own tissues. Such immunization is often quite intense and is frequently accompanied by the production of lesions in the tissue concerned. This type of auto-immunization was first recognized with several protein and polypeptide hormones, most notably insulin. In the last 2-3 years it has been found possible to immunize animals to a variety of tissues in this manner. Thus heart and kidney lesions have been described [2-3]. Hetero-cross-reactive antibodies may show a high degree of specificity, being capable of distinguishing *e. g.* gastric, ileal, and colonic mucosal antigens [4]. While it is improbable that immunization with heterospecific tissue antigen is a common cause of auto-immunization and of disease in man, it is probable that a similar immunization with bacterial or viral antigens cross-reactive with tissue components may occur. A well documented case is the cross-reaction between certain group A streptococcal cell wall antigens and antigens of human heart [5].

4. Introduction into the lymphatic tissues of lymphoid cells derived from an animal of different genetic character may result in the development of a graft-versus-host reaction, the transferred cells being immunized against homograft antigens and other antigens which distinguish them from the host. A variety of lesions may result, some of which are thought to resemble lesions found in the diseases of connective tissue in man [6-7]. It is not clear as yet to what extent the elements of this experiment may be present in any human disorder. Auto-antibodies, against red cells, for example, appear frequently in patients with malignant reticuloses [8]. The possibility that, in such cases, auto-immunization is due to the appearance in the abnormal lymphatic tissues of immunologically competent cells which have lost their host specificity is at present entirely conjectural.

5. Within the last 3 years, two mouse strains have been found which possess a genetic abnormality of the immune apparatus apparently comparable in some degree with that found in human connective tissue diseases such as lupus erythematosus [9-10]. The NZB/B1 mouse and certain hybrids of this strain with other mouse strains develop erythrocyte auto-antibodies, auto-antibodies to nuclear constituents resembling the LE factor, and renal changes comparable to lupus nephritis. The A/J mouse also develops antinuclear antibodies [11]. The nature of the abnormal immune function has not thus far been identified. It may be a hyperreactivity to minimal antigenic stimuli, a deficiency in the function of tolerance, or some other aberrant property. There is some evidence to favor the first of these suggestions [12]. Apparently the thymus is the seat of the abnormality, since this organ in NZB/B1 mice may show ectopic formation of germinal centers and plasma cells [10] and grafting of NZB/B1 thymus to neonatal mice of other strains endows them with the abnormality in question [13]. It is well recognized that lupus erythematosus is one manifestation of a familial disturbance which may give rise to other "auto-immune" diseases, to the formation of various auto-

antibodies, or indeed to disturbances of immunoglobulin synthesis. A relationship between thymus abnormality and certain diseases of this group, notably myasthenia gravis and acquired hypogammaglobulinemia, is fully established. There is an apparent specificity of the genetic disturbance, in that there is little or no overlap in incidence, in families or in individuals, between the connective tissue diseases and myasthenia gravis, on the one hand, and chronic thyroiditis, adrenalitis, and chronic hypertrophic gastritis (with or without pernicious anemia), on the other. The latter form a second constellation of diseases, with overlapping incidence and frequent formation of auto-antibodies but with no known relation to thymus abnormality.

Mechanisms of Lesion Formation.

The theoretical possibility exists that lesions may be produced by any of the following mechanisms: 1) cytotoxic, with direct damage of cells or tissue elements by humoral antibody; 2) direct neutralization, by antibody of physiologically important substances in solution, such as hormones; 3) anaphylactic, with local release of vasoactive agents (histamine, slow-reacting substance, kinins, 5-hydroxytryptamine) secondary to antigen-antibody reaction on or near platelets, mast cells, and possibly other cellular elements; 4) Arthus, with formation of antigen-antibody aggregates in or near vessel walls, local diapedesis of granulocytes secondarily, release of hydrolytic enzymes from lysosomes of these cells, and resulting vascular and tissue damage; and finally, 5) delayed (cellular) sensitivity, with early diapedesis of medium-large lymphocytes in areas containing antigen, transformation of these cells to histiocytes, and damage of parenchyma in the zone of cellular infiltration.

These various mechanisms may all play a role in one or another of the different animal lesions of auto-immunity and the human lesions which resemble them.

1. Cytotoxic lesions are well-known in the case of blood elements, many cases of acquired hemolytic anemia, chronic leukopenia, and idiopathic thrombocytopenic purpura, for example, being directly due to the presence in the circulation of antibody-like globulins of the appropriate specificities. Passive transfer of serum containing "auto-antibody" of this type to normal individuals or passage of such antibody across the placenta results in destruction of cells in the recipient; thus the causative relation of the "antibody" to disease is not in doubt. On the other hand its identification as specific antibody remains uncertain, and the mechanism which results in its formation is not clear.

A wide variety of other "auto-antibodies" is found in man, but their relation to the diseases with which they are associated is in most instances uncertain, since in each case the antibody may be construed either as the cause of the disease or its consequence. In animal experiments, till recently, aside from lesions of blood cells and other cells in suspension (ascites tumor cells), antibody was known to cause

cytotoxic lesions only of vascular endothelium, *i. e.* of cells displayed at a surface. Passively transferred antibody to various solid tissue elements was ineffective in forming lesions (for kidney lesions, see discussion below). Even *in vitro,* cells growing in organized masses appeared inaccessible to antibody. It is possible therefore that human lesions of vascular endothelium or serous surfaces (pericardium, pleura, synovia) will be found to result from the direct cytotoxic action of auto-antibody. Recently the suggestion has been made that cardiac lesions may result from the long-continued presence of antibody to heart muscle in high titer in the circulation [2]. To what extent such a relationship may hold with other solid tissues is entirely conjectural at the present time.

2. Neutralization by antibody of insulin in cases of recurrent hypoglycemia and of intrinsic factor in pernicious anemia appear to be clear examples of auto-immune lesions mediated by antibody, though again, in the latter instance, the genesis of the "auto-antibody" is unclear. Presumably similar lesions affecting other hormones or secreted materials may be identified in due course. A number of animal models exist which confirm the mechanism suggested for the human diseases.

3. Anaphylactic lesions involving blood or tissue antigens and "auto-antibody" have not as yet been identified either in animals or in man.

4. Lesions of Arthus type involving tissue antigens do occur. The character of the nephrotoxic nephritis produced with antisera to glomerular basement membrane antigen suggests that it is correctly assigned to this class of lesions. A recent study [14] has shown that similar lesions may result in vascular or extravascular sites, when antibody directed against one of several connective tissue antigens is introduced. The role of such a mechanism in human disease remains, however, unknown, though "allergic" vasculitis frequently has a morphologic character suggesting an Arthus mechanism.

5. A good deal of evidence supports the suggestion that auto-immune lesions of solid tissues in animal experiments are of the cellular or "delayed" type. This evidence is partly morphologic and rests partly on passive transfer studies with "sensitized" cells. Till recently, no satisfactory *in vitro* technique of studying and identifying this class of reactions was available. Newer modifications of the Rich tissue culture phenomenon [15] and the demonstration in cell culture of a direct cytopathogenic action of "sensitized" lymphocytes on target cells [16, 17] permit a further analysis of the mechanism of formation of this class of lesions. A cytopathogenic effect of specific lymph node cells on peripheral nerve myelin in culture [18] and on thyroid cells [19] has now been observed. There is still no information regarding the mechanism of diapedesis of "sensitized" cells at sites of new lesion formation nor as to the mechanism whereby the cytopathogenic effect is brought about. The role of locally formed antibody also remains to be evaluated. With regard to various solid tissue lesions in man, no positive evidence exists as yet to incriminate the delayed mechanism of lesion formation.

Nevertheless the appearance of the various lesions and the general failure to find any evidence for a pathogenetic role of the "auto-antibodies", with which they are associated, makes this possiblity an attractive one which requires intensive investigation.

In conclusion auto-immune mechanisms have, within a 20 year period, ceased to be an immunologic curiosity and come to be recognized as playing a major etiologic role in many diseases, both of laboratory animals and man. The various conditions under which auto-immunization occurs and the means whereby lesions are actually produced are well on the way to elucidation.

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ALLERGY AND COLLAGEN DISEASES

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Collagen diseases should be termed systemic mesenchymoses or systemic connective-tissue diseases, because collagen is not primarily affected. In this group of diseases, the connective tissue as a biological system is affected, both the cells and the intercellular substances. The etiology of these disorders is unknown and possibly different from disease to disease. In all of them the mesenchyme in the extended sense is damaged, the reticuloendothelial system participating actively in the disease processes.

The tissue changes characterizing all the "collagen diseases" are edema, mucinous edema, serous inflammation, granuloma formation, fibrosis and, possibly, necrosis and calcification. An increased permeability of damaged blood vessels seems to be of primary importance to all the group. Vasculitis is a prominent feature. In the edema are proteins, including enzymes and enzyme inhibitors. The watery edema elicits a mucinous organization, a binding of the water by acid mucopolysaccharides released from tissue mast cells. If the tissue edema persists, a fibrous organization invariably takes place, i. e. a deposition of collagen fibres or cicatrization.

Fibrinoid deposits in the intercellular compartment may be seen in the systemic mesenchymoses. Fibrinoid is not a well defined substance. It may include nucleoprotein, gamma globulin and fibrin. Fibrinoid is found in several allergic conditions and may be induced by injected foreign protein, horse-serum, egg-albumin, meningococcal toxin, E. coli endotoxin, streptococcal filtrates and pleura exudates. However, fibrinoid has also been found in tissues in the course of diseases that have possitively *no* allergic pathogenesis. It should be clear that fibrinoid does not specifically indicate allergic disease.

It is by no means a well-established fact that the systemic connective-tissue diseases have a common or even similar pathogenesis. The connective-tissue response to different damaging agents may be similar as evaluated by histological and biochemical criteria.

Rheumatic fever: It is generally accepted that allergy plays the predominant role in rheumatic fever. Hemolytic streptococci are blamed as the offending etiologic agent. By repeated injections to rabbits of foreign protein, tissue reactions in the myocardium very similar to

Stein-Aschoff bodies have been observed. RICH (1946), finding early fibrinoid changes in the connective-tissue ground substance, and suggesting that histamine might be one of the damaging agents released as a response to an antigen-antibody interaction, characterized the anaphylactic carditis by its similarity to polyarteritis nodosa. Evidence has been presented that streptolysin O may be responsible for rheumatic carditis. The finding of circulating auto-antibodies against myocardial antigens has led several authors to believe and postulate that rheumatic fever is basically an autoimmune disease. KAPLAN (1960), using immunofluorescent technique demonstrated a binding of gamma globulin to the myocardium. He proposed the streptococcal toxins to cause the original cardial damage that, secondarily, start autoimmune mechanisms. A primary synovitis produced by streptococcal toxins might well be the stimulator of autoimmune chain reactions that might affect the endocardium and the serosal membranes.

Rheumatoid arthritis: The serum of many patients with rheumatoid arthritis agglutinate group A hemolytic streptococci and sensitized sheep blood cells. However this serum factor can also agglutinate bacteria, latex or bentonite if coated with gamma globulin or antibody, and also nonsensitized kaolin and colloid particles. The rheumatoid factor has antibody characters. Its sedimentation constant is 19 S. It combines with ordinary 7 S globulin forming a complex with a sedimentation constant of 22 S. The rheumatoid factor is believed to be formed by plasma cells. Glynn et al. (1957) suggested that the rheumatoid factor is an antibody directed against antigenic determinants shared by the gamma globulin of various species. The rheumatoid factor and the reactant do not fix complement. The factor is present in the serum of 85-90 per cent of patients with rheumatoid arthritis, and some authors require a positive serological reaction to establish the diagnosis. The serum titer does not convincingly parallel the intensity of the rheumatic disease. Injected into human individuals it does not aggravate an existing disease and cannot produce symptoms in normals. Relatives to patients may carry the serum factor without displaying any clinical signs of disease. In rheumatoid arthritis patients, the L. E. factor as well as leucocyte and platelet antibodies have been demonstrated.

The fluorescent antibody technique has shown that mononuclear lymphocytoid cells of the synovial villi synthesize rheumatoid factor. Plasma cells have been found to contain the factor, but it must be emphasized that rheumatoid arthritis may occur in the serum of patients with agammaglobulinemia. Denatured gamma globulin has been demonstrated in rheumatoid tissue lesions.

An autoimmunization may be active in rheumatoid arthritis. However, there is evidence that this is secondary, while the primary factors are unknown. Trauma, including surgical operations, may be counted to them.

MACKAY and BURNET (1964) believe that an immunological defect exists in sufferers from rheumatoid arthritis, and that this defect is in some way responsible both for the development of rheumatoid

arthritis and immune reactivity to gamma globulin, as manifested by the rheumatoid factor in the serum.

Lupus erythematosus: There is evidence that patients developing lupus erythematosus are in some way predisposed. Characteristic pathological lesions are vasculitis with fibrinoid necrosis, lymphocytic infiltration and granuloma formation. Hematoxylin stainable bodies have only been found in systemic lupus erythematosus (SLE). These amorphic masses containing DNA may be phagocytized by histiocytic cells. The L. E. factor belongs to the gamma globulin fraction of serum. It has antibody characters reacting with blood cell nuclei, which may swell, lose their basophilia and their characteristic chromatin structure. They may then be phagocytized by vital leucocytes forming L. E. cells. The L. E. factor may react with nucleoprotein from various species, mammalian, avian, and bacteria as well. Thus, there is a notable lack of specificity. A great number of other antinuclear and, in addition, anticytoplasmic factors have been revealed in SLE. Such antibodies are directed against mitochondria and microsomes. Antibodies against leucocytes and platelets have been described.

L. E. may be accompanied by hyperglobulinemia, hemolytic anemia, a positive Coomb's test, thrombocytopenic purpura, Wassermann and Kahn reactivity, and hemorrhagic phenomena due to circulating anticoagulants.

Certain drugs, i. e. hydralazine, penicillin, and others, have been reported to produce L. E.-like disease. Cold and sunlight may also act as precipitants, preferably in predisposed individuals.

Bacterial, preferably streptococcal, and virus infections have been blamed as noxious stimuli. Nuclear components of bacteria may be antigenic and there are undoubtedly some common features in SLE and rheumatic fever. However, there is no conclusive evidence to support such speculation.

The overwhelming predominance of disease among young women in their reproductive age has suggested that endocrine factors might be active. Estrogenic hormone may have a conditioning effect, but there is no evidence that hormonal factors are of primary significance.

Polyarteritis nodosa: In polyarteritis nodosa the cutaneous bleedings and necroses, the erythema, papules and urticarial wheals are results of connective-tissue changes in the vessels. Endothelial damage and granulation tissue in the vascular walls may entail thrombosis and occlusion of the lumen and the formation of small aneurisms. The deeper cutaneous and subcutaneous arterioles are regularly involved as well as the arterioles of the gastrointestinal tract, the heart, kidneys and the lungs.

The pathological changes are very similar to those of *serum sickness.* There is much evidence that an antigen-antibody reaction is active. Polyarteritis nodosa-like changes may be produced by injection of foreign protein, by administration of drugs of various kinds, corticotrophin, sulfonamides, and others.

The pathological changes in *Schönlein-Henoch's purpura* are also

similar to polyarteritis nodosa. It is a generalized vascular disease with fulminant symptoms which may be provoked by drug or food allergy.

A relationship possibly exists to rheumatic fever, which may accompany polyarteritis nodosa. Polyarteritis nodosa may be preceded by streptococcal infection. Other allergic diseases such as asthma, angioneurotic edema and urticaria occur relatively frequently in patients with polyarteritis nodosa and in their relatives.

Polyarteritis nodosa patients have hypergammaglobulinemia, but no autoantibodies have been demonstrated yet. There is no conclusive evidence of autoimmunization being active in polyarteritis nodosa.

Scleroderma: In both the generalized and localized types of scleroderma, there is atrophy of the epidermis and hypertrophy of the dermal connective tissue. The sclerotic fibrosis also involves the subcutaneous layer as well as all connective tissues of the body. Of special interest are the vascular walls, the myocardium, the lungs, the pancreas, and the liver. Fibrinoid and amyloid changes as well as calcification may occur.

Whether of primary or secondary significance, the changes in the vessel walls play a special role in the pathogenesis. The histological background of the vasospastic crises is a subacute arteritis including thickening of the intima, fibrosis of the walls and partial or complete obliteration. The small and medium sized arteries are preferably affected. The vascular changes are predominant in the generalized type of scleroderma, whereas in the localized types the extravascular damage seems to be prevailing.

Scleroderma patients may show hypergammaglobulinemia and circulating autoantibodies in the serum (red cell agglutinins, L. E. factor, Wassermann-like antibodies). A circulating antinuclear globulin reacting with autologous and homologous as well as heterologous tissues has been demonstrated in cases of generalized scleroderma. The reaction may be inhibited by desoxyribonuclease.

The development of scleroderma has been observed following vaccine and serum therapy, but there is still no evidence that allergy or autoimmunization play a pathogenetic role in scleroderma.

Dermatomyositis: Dermatomyositis may be localized or involve all the skin and several muscle groups. The increased capillary permeability, the mucinous edema and inflammation are the predominant features. In early and fulminant cases the changes may be similar to L. E., whereas in more protracted cases they may be very like scleroderma. Calcinosis is not rare. The tender, swollen, edematous muscles of the acute phases and the atrophic fibrotic muscles of the chronic reflect the involvement of the mucinous and collagen apparatus, respectively.

A dysproteinosis with hypergammaglobulinemia is regularly seen. An antinuclear globulin has been demonstrated by the fluorescent antibody technique. About 15 per cent of dermatomyositis cases are associated with cancer. The symptoms may precede the signs of the

malignant tumor. There is some evidence that the disease may develop as an allergic response to tumor products.

Conclusion.

— The systemic mesenchymoses may be the result of the damaging effects of an immune response directed against an *exogenous* antigen, possibly specifically against a hapten.

— The antigen may be an auto-antigen and *not* foreign.

— The immune phenomena may be accompanying the disease without any pathogenetic significance.

— Hypersensitivity to exogenous antigens can not explain the persistence of these diseases. On the other hand, evidence for an auto-immune mechanism is scarce and inconclusive.

THE THYMUS AND AUTOIMMUNITY (*)

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There are, perhaps, no two concepts that have greater currency in contemporary immunology than "thymus" and "autoimmunity". Both the idea of an immunologic role for the thymus and the concept of "autoaggression" have long histories; but, interestingly, clinical and experimental investigations in both areas have reached a peak during the last three or four years. A wide range of opinion exists on the degree of interrelationship of the thymic role in the body economy and the group of serologic and pathologic phenomena encompassed by "autoimmunity" —from the assertion that the thymus plays a causal role in these conditions at one extreme, to the suggestion that the thymic lesions—and, indeed, many of the abnormal antibodies and other autoimmune phenomena—reflect tissue damage that is not fundamentally immunologic at all.

Thymic Function in the Mouse

Until very recently, research on the immunologic role of the thymus emphasized the ontogenetic development of the immune mechanism. This, certainly, was the central concept in the earliest work of ARCHER *et al.* [1-2] on the rabbit; of MILLER [3-4] and MARTÍNEZ, GOOD *et al.* [5-6] in mice; and of WAKSMAN and associates [7-8] in rats: that the thymus plays an essential role at about the time of birth in these species in both the structural development of the lymphoid tissues and the maturation of immunologic capability, particularly antibody producing capacity and transplantation immunity. Deprived of their thymuses in the newborn period, these animals suffer varying degrees of immunologic deficiency, usually extreme in mice, less so in rats, and much less so in rabbits.

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This early view of the function of the thymus has been broadened and refined in several directions. It is known that the thymus' influence on immunologic development in the mouse continues to 40 days of age and beyond [6, 9]; and that its control of lymphoid tissue integrity and immunologic competence is reasserted in the fully grown animal if it is later exposed to high doses of irradiation, depleting the lymphocyte population and the level of immune reactivity [10-12] or if immunologic responsiveness is specifically suppressed in an immunologic tolerance model [13].

It is known, too, that in the newborn mice the thymic effect on the immune mechanism is exercised by a humoral, presumably hormonal factor, at least in part; two groups of investigators [14, 15] have shown that neonatal thymic tissue, contained in cell-impermeable diffusion chambers, offers partial restoration of lymphoid tissue development and immunologic capability to the neonatally thymectomized mouse. A third group has demonstrated an analogous effect in neonatally thymectomized rats similarly grafted [16].

It seems probable to us that the role of the mouse thymus is, in the late embryonic and early postnatal period, partly cellular. This idea of the thymus as a central source for cells that are distributed centrifugally to the peripheral tissues was expressed as early as the 1890's by BEARD [17]; modern students of the thymus, FICHTELIUS [18], AUERBACH [19] and RUTH [20], among them, have, from different lines of evidence, formulated similar hypotheses; and these have figured prominently in the speculation on the nature of the deficiency in neonatally thymectomized mice. It was clear, in initial studies, that adult spleen and lymph node cells would prolong life and restore immunologic function to neonatally thymectomized mice if administered before they entered their period of physiologic decline [9, 21-22], the so-called wasting disease which quite consistently develops in most strains of inbred mice deprived of their thymuses in the immediate neonatal period. Thymocytes proved ineffective in the early studies, work involving, by present standards at least, the injection of relatively small numbers of cells; however, TAYLOR [23] and YUNIS, HILGARD and associates [24-25], in our laboratories, have since shown that thymocytes, even from neonates, will avert the wasting syndrome and restore immunologic function to the thymusless animal so that it will produce antibody and reject allogeneic (from another strain) skin grafts. The lymph nodes and spleen, which in wasting animals are atrophic, poorly structured, and sparsely populated with lymphocytes, are apparently restored by the injected cells; if these tissues from the "salvaged" animals are injected into appropriate neonatal hybrid mice they show activity against the host typical of the strain of the thymocyte *donor* rather than the thymectomized recipient [24-25]. Even more recent studies, involving administration of huge numbers of newborn thymocytes to allogenic thymectomized hosts, suggest that these inocula may also foster immunologic development of *host* cells as revealed in assays of graft versus host activity [26].

Thus—and we shall return to this concept later—the evidence sug-

gests that the immunologic and physiologic failure of neonatally thymectomized mice is directly correlated with the poverty of the other lymphoid tissues, particularly such "effector" organs as lymph nodes and spleen .These tissues can be restored either by supplying the facilitating influence of humoral substances from thymic tissue in diffussion chambers, by repopulating the tissues with injected cells—either from peripheral lymphoid tissues or, in larger numbers, from the thymus—or by providing a thymus graft whose contribution to the thymus-deprived mouse is apparently both humoral and cellular.

There is, perhaps, no more fascinating clinical parallel to the neonatally thymectomized mouse (and the neonatally thymectomized hamster, which is also very deprived immunologically and prone to develop a severe wasting syndrome) than infants with the Swiss type of agammaglobulinemia [27-34]. These children have less immunologic capability than any other defined patient group: they are very susceptible to infection with the whole range of micro-organisms, show defective delayed allergy and homograft immunity, often have malabsorption syndromes and other digestive disturbances, usually show growth failure and, in the late stages, a wasting process. Their thymuses have invariably been abnormal in size and structure: ranging from tiny vestiges of tissue, a few milligrams in some instances, located in the thyroid area; to very small non-lymphoid organs in the normal intrathoracic position; and, at the other extreme, tissues of several grams in weight but entirely epithelial and stromal in composition. As in the neonatally thymectomized mouse, the other lymphoid tissues have been very small, lacking separation of cortex and medulla, and almost completely devoid of lymphoid cells. The parallel to the neonatally thymectomized hamster is better in one dimension: wasting hamsters are gamma globulin-deficient [35], as are the Swiss agammaglobulinemic children; the neonatally thymectomized mouse sometimes has a low gamma globulin level, but usually does not [22].

The Bursa of Fabricius and Appendix.

In two species, the chicken and the rabbit, it has been shown that the thymus is one of two—perhaps more—"central lymphoid tissues": organs which influence immunologic maturation and lymphoid tissue development, while taking what seems to be a less active role in day-to-day encounters with antigens and other forms of "foreignness". In the chicken, the organ is the bursa of Fabricius, sometimes called the cloacal thymus, and its role seems to be limited largely to the mechanism for production of circulating antibody [36-38]. The role of the chicken thymus is not as well defined, but evidence from several laboratories suggests that its influence may be limited to cellular immunity [39-42].

The rabbit's lymphoid system is more complex; to the present time, we have demonstrated a role in the maturation of antibody producing capability and development of the lymphoid tissues and cell systems for both the thymus and the appendix [1-2, 43-45]. No effect has yet been shown

on delayed hypersensitivity or homograft immunity [46]. We have evidence that there are other "central lymphoid tissues" in the rabbit, and experiments aimed at their definition are proceeding. The tissues involved are probably the intestinal tonsil (sacculus rotundus), and, possibly, certain Peyer's patches and even the pharyngeal tonsils and adenoids —all the "central lymphoid tissues" seem to be associated with the gastrointestinal tract and may have their developmental origin in epithelium.

Recent findings on the role of "central lymphoid tissues" in certain malignant developments serve to strengthen this concept. It has been known for 20 years that early thymectomy of mice greatly reduces the incidence of lymphatic leukemia in that species, an observation originally made in the high leukemia strain, AK [47], but extended in later years to lymphomas induced by radiation, virus and carcinogens [48-50]. A parallel has recently been found in two other species, the rat and the chicken. In the rat, early thymectomy apparently reduces the incidence of Gross-virus-induced lymphomas, although not as dramatically as it does in the mouse [51-52]. On the other hand, in the chicken, bursectomy reduced the incidence of a virus-induced visceral lymphomatosis to almost nothing, without affecting the mortality from other neoplasms associated with the well characterized filtrate used [53].

We have, then, a parallel in three species of a significant influence of central lymphoid tissue on antibody producing capability and on lymphomagenesis.

There are two series of clinical observations which have come to the fore in recent months as we have considered the implications of these recent experimental findings for the ward and clinic and, indeed, the reciprocal influence of clinical experience and observation on laboratory animal studies.

The first is a clinical reinforcement of the triad just mentioned: central lymphoid tissue, immunologic deficiency, and lymphocytic malignancy, occurring in children with ataxia-telangiectasia [54]. This disease, originally described by LOUIS-BAR [55], is usually manifest in early childhood: the cerebellar ataxia is in most instances the earliest sign, followed by appearance of the telangiectases of the sclera, eyelids and ear lobes, and, in most of the patients, by unusual susceptibility to respiratory infection [55-56]. Many of the children have not been studied for immunologic deficiency or for abnormalities of the immunoglobulins; but, of those that have, almost all showed some abnormality of immunologic function (particularly of homograft immunity and delayed hypersensitivity) [54] and many had deficiencies of one or more of the immunoglobulins, the gamma^{A} (beta$_{2A}$) most often, but also the gamma^{G} (gamma$_2$) in some instances [54, 57]. Where the gamma^{G} has been low, there have been corresponding deficiencies in antibody responses to strong antigens [54, 58].

This patient group has two additional characteristics which place it into extraordinary perspective in view of the known relationships of central lymphoid tissue to both immunologic development and lymphomagenesis in certain experimental animals: these are the gross abnor-

mality, or absence, of thymus tissue in virtually every patient in whom thymus has been sought at postmortem, or studied following biopsy or autopsy [54-55]; and the unusually high incidence of reticular and lymphocytic malignancy [54]. The total number of cases of this syndrome in the literature and known to us by personal communication is about 125: of this number at least 8 have died of lymphoreticular neoplasms, 4 have had no thymus tissue at autopsy, and 3 studied by us at autopsy or biopsy had very small thymuses, entirely lacking in normal structure, and very sparsely populated with lymphoid cells. This tissue resembled embryonic thymus, the epithelial rudiment, at the very beginning of lymphoid differentiation [54].

The second series of observations concerns sex-linked recessive agammaglobulinemia, a syndrome associated with very low gamma globulin levels and extreme immunologic deficiency, particularly of circulating antibody production. We have had an opportunity to study thymus tissue of five such patients; except for absence of Hassall's corpuscles in the sections available in one case, and a degree of lymphocyte depletion consistent with the infections the children suffered from, these thymuses were normal [34]. The other lymphoid tissues of these children have been studied: lymph node, spleen, lymphoid tissue of the appendix and Peyer's patches—and have consistently been small, poorly structured, entirely lacking in follicular development, and poor in lymphocytes [62]. In the light of the contrast with the Swiss agammaglobulinemia patients, mentioned earlier, and the analogy to the bursectomized chicken—also deficient in gamma^G globulin, plasma cell formation, and antibody producing capability [36-38, 40, 63, 64]—we have speculated that a degree of dissociation of lymphoid tissue function may characterize the human immune mechanism, and that the extreme deficit of the gut-associated lymphoid tissues, other than the thymus, may underlie the immunologic failure of the congenital sex-linked agammaglobulinemic.

Two other elements in the history of this patient group also warrant mention here: at least three boys with this disease have developed lymphoma or leukemia, one of whom presented with a large thymoma [65]; and many have suffered from such mesenchymal diseases as rheumatoid arthritis, scleroderma, and dermatomyositis, diseases associated with abnormal antibody formation in persons with normal gamma globulin levels [66-68].

In summary, then, we have the developing concept of central lymphoid tissues, apparently originating in gut epithelium, and exercising crucial influences both in the maturation and maintenance of lymphoid tissues and adaptive immunity, and in the development of certain leukemias, lymphomas, and reticular tumors.

Autoimmunity in Mice.

As indicated earlier, experiments in inbred mice have been central to the developing knowledge and understanding of the thymus. There is another strain, NZB/BL, developed in New Zealand by BIELSCHOWSKY

et al. [69], that has been the subject of a series of provocative studies of the thymus in relation to autoimmunity. These animals regularly develop a Coombs-positive anemia [69] and thymic lesions of the germinal center type [70]. Two hybrids of this strain have been described (NZB/BL × NZY/BL) and (NZB/BL × NZW); both often develop a lupus-erythematosus-like syndrome featured by "lupus nephritis" and positive LE tests [71-72]. Most extensive studies have been done with the second hybrid which develops the disease earlier and in much higher incidence.

HELYER and HOWIE [72] and BURNET and HOLMES [73] have studied the effects of splenectomy, thymectomy, and thymic replacement in these animals. Early splenectomy and ACTH therapy delayed onset of the hematologic disease in NZB/BL [74]; but thymectomy seemed to result in an earlier, more severe disease [72]. Of greatest interest were the thymic replacement studies [72]: thymuses from both the NZB/BL and (NZB/BL × NZW) hybrid thymuses were grafted into CBA/T6 animals whose own thymuses had been removed immediately after birth; and normal CBA/T6 thymuses were grafted into neonatally thymectomized NZB/BL and hybrid animals. The results in the studies of Helyer and Howie were clear-cut: grafting of a normal thymus into the autoimmune strain or its hybrid had no ameliorating effect. Indeed, the disease was earlier in onset and more florid in its manifestations than in the nonthymectomized groups. On the other hand, when thymus tissue from NZB or (NZB × NZW) animals was grafted into thymectomized CBA/T6 mice, about half of the CBA mice developed one or more manifestations of the disease process.

Although it is reported that detailed immunologic studies of the NZB mice are underway [75], no data are available at present regarding the immunologic status of these animals other than the Coombs positivity.

The immunologic status of the NZB mice is of particular interest because of the recent suggestion of DEVRIES et al. [76, 77] that certain strains of neonatally thymectomized mice develop Coombs positivity and a syndrome very reminiscent of certain graft-versus-host reactions. The analogy of the wasting disease of neonatally thymectomized mice to various forms of homologous and secondary disease has been commented on repeatedly. Since both lymphoid tissue atrophy and immunologic deficiency are characteristic of mice with all three of these "wasting" syndromes, the issue of neonatal thymectomy as cause, and lymphoid tissue defect and immunologic depression as effects, has never been satisfactorily resolved. Our own experience has been that some neonatally thymectomized mice of certain strains do not develop wasting disease, but are as deficient immunologically as the runting animals, although their lymphoid tissues may not show as extreme defects. Coombs positivity has also been a rare finding in the neonatally thymectomized mice we have studied. We have, however, observed intestinal crypt lesions and mild amyloidosis in some of the neonatally thymectomized mice [78] and intestinal crypt lesions in neonatally thymectomized hamsters [79].

This thesis of "autoimmune" mechanisms as etiologic factors in

the physiologic decline of neonatally thymectomized mice warrants serious consideration, bolstered as it is by the evidence of DEVRIES et al. [76, 77], by some of the data of PARROTT and EAST [22], and by certain phases of the general experience with these animals. It has been given further support recently by a model developed in immunologically deficient thymectomized and appendectomized rabbits (see below).

Thymic Abnormalities in Myasthenia Gravis and Systemic Lupus Erythematosus.

BURNET and HOLMES [70, 73] have emphasized the similarity of the thymic lesions in NZB mice to those characterizing many myasthenia gravis patients, as well as a parallel, in some instances at least, to the preleukemic thymic changes in the high leukemia strain AKR. The analogy to myasthenia gravis has been strengthened by a series of findings, beginning with those of STRAUSS et al. [80] that many myasthenia gravis patients have antinuclear or antimuscle antibodies or both [81-84]. The work of MARSHALL and WHITE [85] has suggested that the alteration of the normal thymus's relative impermeability to parenterally administered antigens may play a role in the thymic lesions of myasthenia gravis. They showed that direct injection of antigen into the thymus of guinea pigs or intravenous injection of antigen following injury of their thymus, results in lesions reminiscent of those of myasthemia gravis patients. It has become evident in a series of recent studies that the isolation of the thymus from antigens has probably been exaggerated [86-88]. Critics have emphasized the lack of specificity of the thymus lesion, which has been reported in diseases as disparate as Addison's disease [89] and chronic hepatitis [90]; and the similarity of the thymic lesion to that which occurs in such non-lymphoid tissues as heart muscle when antigen is injected directly [91].

The serologic parallel of myasthenia gravis and systemic lupus erythematosus has encouraged the search for other resemblances. A number of patients have been reported in whom both diseases occurred simultaneously (reviewed [84]). Thymomas, which occur in about 15 per cent of myasthenia gravis patients [92], have also been reported in systemic lupus erythematosus [93-94]; and MACKAY and DEGAIL [95] and HARVEY [96] have reported a total of 5 SLE patients, not treated with steroids, whose thymuses had lesions of the myasthenia gravis type. There is at least one additional report of germinal center-like lesions in the thymus of a lupus patient given heavy doses of prednisolone [97].

The efficacy of thymectomy for relief of myasthenia gravis patients was in dispute for many years; but the recent consensus seems to be that significant improvement occurs following thymectomy in many young women with a short history of the disease [98]. In some of them, dramatic results have followed removal of the thymus during a myasthenic crisis.

The issue, both of thymic lesions and of effect of thymectomy, is much cloudier in the case of systemic lupus. Few patients have been

thymectomized, and even fewer have been followed for any extended period. There have been suggestions that some patients have benefited, while others have not [99-101]. It is of interest, however, that several patients with myasthenia gravis have developed full-blown lupus some time following thymectomy [102-103].

There is another group of clinical phenomena, associated with thymic tumors, which warrants mention. Four or five patients have been reported who had myositis, myocarditis, and thymoma, in the apparent absence of myasthenia gravis [104-108]; one of them had a positive LE cell test, but did not have pathologic findings of lupus erythematosus [108]. There have been at least two instances of dermatomyositis and thymoma [109-110]. Most recently, KLEIN et al. [111] reported two myasthenic patients with thymoma and polymyositis, one with hypergammaglobulinemia.

In summary, clinical evidence continues to reinforce the association of certain types of thymic pathology with disease states often termed "autoimmune". This is not to say that they are causally linked or, indeed, that either is of etiologic importance in the diseases under consideration. Much additional study, both with experimental models such as the disease of NZB mice and their hybrids, and with these several patient groups, is needed, along with more critical attitudes on the subject of autoantibodies in particular, but also on thymic pathology.

Thymoma and Hematopoietic Failure: Autoimmunity?

Experimental and clinical data and speculation have inevitably linked the thymus to the lymphoid tissues, the lymphoid system of cells, and lymphoreticular malignancy. Experiments have repeatedly emphasized, for example, that thymectomy has no influence on myeloid leukemias in mice. The many studies involving thymectomy of young or adult mice or rats have shown lymphoid tissue depletion and reduced numbers of circulating lymphocytes, but reports of effects on other hematopoietic cell lines have been rare. Thus, the repeated association, in at least 40 patients, of thymoma with either red cell agenesis or pancytopenia has been a puzzle. These anemias and pancytopenias have proved very resistant to treatment; removal of the tumor has often been ineffective, but most analysts of the data believe that recovery has followed this procedure in a significant number of instances (reviewed [112]).

Recently, two groups, in England [113] and in Denmark [114], have suggested that these syndromes may have an immunologic basis, citing the presence of abnormal antibodies in such patients. Except for the instances in which the hematopoietic failure of thymoma patients has included plasma cells, with resulting agammaglobulinemia, this patient group has not been studied immunologically until recently. Further investigations of this type will be of great interest.

Autoimmunity and Lymphomagenesis.

The relationship of "autoimmunity" to lymphomagenesis has not been adequately explored; this question has been discussed by Dameshek on several occasions [115–116], as well as in more recent speculations of BURNET and HOLMES [70–73].

The relative roles of the "inducer" —virus, irradiation, hormones, carcinogens— and the host facilitating influences, particularly thymic function, have had a great deal of attention in studies of mouse leukemia. The work on drug-induced systemic lupus erythematosus, a disease apparently characterized by typical SLE serology and renal lesions, has suggested that an "inducer" is involved in some, if not all, SLE patients [117]. Perhaps there is also a thymic factor in host susceptibility here. Extensive experience with agammaglobulinemia-hypogammaglobulinemia patients has led to the inescapable conclusion that such patients have an unusual predisposition, if not to SLE itself (the sex distribution of SLE patients and of agammaglobulinemic children may be a factor here), then to rheumatoid arthritis, dermatomyositis, and scleroderma [66–68]. They are also more susceptible to development of lymphoma and leukemia than immunologically normal children [65]. We have suggested before that inability to resist virus infections or failure to cope adequately with aberrant cells could account for the high rate of neoplastic development in agammaglobulinemic children [65]. A similar pair of hypotheses might well be considered as a basis for the unusually high incidence of mesenchymal diseases in immunologically deficient patients. One of the patients in our series who developed lymphoma had dermatomyositis as well as extreme hypogammaglobulinemia [65].

From the data available it is difficult to say whether NZB mice have an unusually high incidence of lymphocytic and reticular tumors; that these do occur has been noted by BURNET and HOLMES [73]. They have also noted similarities in some of the thymic lesions of NZB animals and mice of the high leukemia strain AKR.

Autoaggression in Thymectomized-Appendectomized Rabbits.

The earliest demonstrations of an immunologic role for the thymus were the studies of FICHTELIUS et al. in the guinea pig [118] and of ARCHER et al. in the rabbit [1–2]. Later work showed that the rabbit appendix plays a coordinate role in the development of the lymphoid tissues and of antibody producing capability in the rabbit. Rabbits thymectomized and appendectomized in the newborn period have immature, underdeveloped lymph nodes and spleens, and low levels of lymphocytes both in tissues and in the circulation well into the fourth month of life; and their response to a serum protein antigen is very inadequate. The definition of the immunologic deficit in these animals is incomplete, but it appears in preliminary work that homograft immunity and the reactions of delayed hypersensitivity are relatively intact.

One of the remarkable developments in this experimental group is the relatively high incidence (perhaps half of the animals) of a syndrome characterized by a Coombs positive anemia, with diarrhea [119]. Such developments were also noted in isolated rabbits subjected to a single procedure, either thymectomy or appendectomy. One thymectomized rabbit developed extensive amyloidosis involving spleen, nodes, kidney and liver.

We noted briefly earlier that mice thymectomized as adults and heavily irradiated show very poor recovery of the lymphoid tissues and of immunologic capability. ARCHER et al. [120] also showed that this applies to the antibody response of rabbits thymectomized and irradiated as adults. In more recent work [121] rabbits were thymectomized or appendectomized or both as adults and then irradiated. Coombs positivity was found in all three groups, particularly those subjected to both procedures; and amyloid disease developed in some of the thymectomized mice and in approximately half of those thymectomized and appendectomized. Of great interest was the abundance of plasma cells in the depleted nodes and other lymphoid tissues. The syndrome was also characterized by wasting reminiscent of homologous disease or secondary disease of radiation chimeras. Amyloid accumulations are also characteristic of these two groups and of parabiotic mice and hamsters [122-124]. In this connection, MICKLEM [125] has recently reported that amyloid lesions occur with significant frequency in lethally irradiated animals restored with an inoculum of bone marrow sufficient to save the animals but insufficent to restore the lymphoid tissues.

Thus, we have, in the appendectomized-thymectomized-irradiated rabbit, immunologic deficiency, failure of lymphoid tissue recovery, physiologic decline and wasting, and amyloid lesions, offering remarkable parallels to a host of "runting" syndromes induced by various means, including thymectomy in certain strains of mice.

SUMMARY.

Many recent data have provided support for the concept of "central lymphoid tissue" —organs which seem to have their origin in specialized lymphoepithelial tissue of the gut, and which exercise humoral, and probably cellular, influences on the normal maturation and maintenance of the peripheral lymphoid tissues, the development and sustenance of adaptive immunologic capacity, and the abnormal proliferations of at least some lymphoreticular malignancies. The apparently spontaneous occurrence of "autoimmune" phenomena, serologic and pathologic, in immunologically deficient thymectomized (or thymectomized and appendectomized) animals suggests disruption of immunologic recognition mechanisms. Alternatively, they may be more susceptible to viruses or mycoplasma infections— the role of such agents in this group of diseases is still an open question.

Several provocative clinical models have provided significant insights into the function of the "central lymphoid tissues" in developing and

maintaining adaptive immunity, and their dysfunction in immunologic deficiency diseases, myasthenia gravis, and possibly systemic lupus erythematosus and other mesenchymal diseases.

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ALLERGIE ET HEMOPATHIE

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Par hémopathie "allergique" on entend les cytopénies provoquée par un anticorps actif seulement en présence d'un produit exogène, médicamenteux le plus souvent.

L'atteinte peut porter sur l'une des trois lignées du sang, les érythrocytes, les leucocytes ou les plaquettes, et rarement à la fois sur deux de ces lignées.

Les caractères d'apparition de ces cytopénies sont trop connus pour y insister à nouveau [1]. Rappelons simplement:

a) La nécessité d'un contact préalable avec le produit. La *dose sensibilisante* peut avoir été prise très longtemps avant l'accident ou dans les quelques jours qui le précèdent. De toute façon un délai de 5 à 9 jours entre la dose sensibilisante et la dose déclenchante est indispensable pour l'élaboration de l'anticorps et son élévation à un titre suffisant.

b) La nécessité d'une *dose déclenchante* qui peut être minime.

c) La brutalité de l'accident: anémie hémolytique sévère, purpura thrombopénique alarmant ou agranulocytose, rarement annoncé par un accident mineure à la prise précédente.

d) La rapidité de la guérison si tout contact avec le produit a cessé. Dans les cas typiques la destruction est périphérique, la moelle intacte et la repopulation se fait dans un délai de quelques jours.

e) La rechute certaine et généralement plus grave en cas de nouveau contact avec le même produit ou parfois avec un produit voisin réagissant également avec l'anticorps.

Pour arriver au diagnostic exact il faudra souvent procéder à une enquête minutieuse voire policière pour rechercher le produit responsable parmi tous les médicaments administrés qui sont souvent des médicaments composés; ou parmi les produits avec lesquels le malade peut avoir été mis au contact dans sa vie professionnelle.

Il faudra enfin éliminer les autres cytopénies d'origine médicamenteuse en particulier celles qui peuvent apparaître chez les individus dont les hématies sont déficientes en glucose-6-phosphate deshydrogénase, ou présentent une hémoglobine anormale (H, Zurich).

Anticorps "allergique" anti-P.A.S.

Etude des radicaux actifs (I)

POSITIFS			NÉGATIFS		
MÉDICAMENTS	FORMULE	RÉACTION	MÉDICAMENT	FORMULE	RÉACTION
Para-amino-salicylate de sodium	COO Na, OH, NH_2	++++ 1/64	Benzoate de sodium	COO Na	—
3-amino-salicylate de sodium	COO Na, OH, NH_2	++ 1/2	Phtalate de sodium	COO Na, COO Na	—
5-amino-salicylate de sodium	COO Na, OH, H_2N	++ 1/8	Para sulfo-salicylate de sodium	COO Na, OH, HO_3S	—
Para-amino-benzoate de sodium	COO Na, NH_2	++++ 1/64	Acétyl-salicylate de sodium	COO Na, $OCOCH_3$	—
Salicylate de sodium	COO Na, OH	+++ 1/16	Para-amino-hippurate de sodium	CO NH CH_2 COO Na, NH_2	—
Gentisate de sodium	COO Na, OH, HO	+++ 1/32			
Méthoxy-2-benzoate de sodium	COO Na, OCH_3	+++ 1/8			
Nitro-3-salicylate de sodium	COO Na, OH, NO_2	+ 1/2			
Nitro-5-salicylate de sodium	COO Na, OH, O_2N	+ 1/2			

Tableau 1.—Exemple d'étude des radicaux nécessaires à la réaction d'un anticorps allergique anti-PAS (2). Le noyau benzenique et le radical COONa en position 1 sont nécessaires mais non suffisant. Un deuxième radical est indispensable qu'il s'agisse de OH ou OCH_3 en position 2 ou de NH_2 en position 4.

Les allergènes.

Devant l'incertitude du rôle exact du produit dans le déterminisme immunologique des accidents, nous préférons désigner les agents chimiques incriminés sous le terme d'allergène qui est ainsi substitué à celui d'haptène dont la signification immunologique est bien précise.

Il est impossible à l'heure actuelle de trouver une dénomination

ANEMIES HEMOLYTIQUES ET CHOCS AU PAS

MEDICAMENTS	FORMULES	Mac GIBBON	Van LOGHEM	DAUSSET ET GUIMBRETIERE				
PAS	H_2N-C$_6$H$_4$-COOH, OH	+	+	+	+	+	+	+
AMINO-BENZOATE	H_2N-C$_6$H$_4$-COOH	+		+	+	-	-	-
SALYCILATE	C$_6$H$_4$-COOH, OH	+		+	+	+	+	+
3-AMINO-SALYCILATE	H_2N, C$_6$H$_3$-COOH, OH			+	-	-	+	+
5-AMINO-SALYCILATE	NH_2, C$_6$H$_3$-COOH, OH			+	-	-	-	-
GENTISATE	HO, C$_6$H$_3$-COOH, OH			+				
METHOXI-2-BENZOATE	C$_6$H$_4$-COOH, OCH_3			+	-	-	-	+
NITRO-3-SALYCILATE	NO_2, C$_6$H$_3$-COOH, OH			+	-	-	-	+
NITRO-5-SALYCILATE	NO_2, C$_6$H$_3$-COOH, OH			+	-	-	-	-

Tableau 2.—Réactions croisées observées avec des anticorps allergiques anti-PAS. Cinq anticoprs provenant de 5 malades différents ont été étudies par DAUSSET et THIERFELDER (3). Ils montrent, à titre égal, la diversité de ces réactions croisées.

commune aux différents produits qui se sont montré responsables d'accidents cytopéniques d'origine allergique. Ils appartiennent à des familles chimiques très diverses.

Cependant leur poids moléculaire est habituellement faible (malgrè certaines exceptions comme par exemple la pénicilline). Ils présentent fréquemment des radicaux connus pour leurs liaisons faciles. Enfin ils présentent pour la plupart un noyau benzénique, mais c'est aussi le cas de nombreux autres médicaments.

Il n'est pas impossible que plusieurs d'entre eux aient un produit commun de métabolisme expliquant leur action néfaste.

Il est également intéressant de noter que leur fixation à la cellule, en l'absence de l'anticorps peut être labile (sédormid, quinine, quinidine) ou au contraire ferme (pénicilline).

Dans la grande majorité des cas l'anticorps est spécifique pour un produit donné. Cependant dans d'autres cas on a pu observer des réactions croisées avec des corps très voisins du produit ayant provoqué la sensibilisation. A l'aide de ces réactions croisées on peut obtenir quelques renseignements sur la nature et la position des radicaux in-

SPECIFICITE DU SUPPORT ERYTHROCYTAIRE

TYPE D'HEMATIES	PAS 1er cas	PAS 2e cas	PHENACETINE
HEMATIES HUMAINES			
NORMALES	+	+	+
TRYPSINISEES	+	+	+
TANNEES	-	-	-
FORMOLEES	-	-	-
HEMATIES D'AUTRES ESPECES			
LAPIN	-	-	-
MOUTON	-	-	-
COBAYE	-		
POULE	-		

Tableau 3.—Etude du support cellulaire à l'aide de deux anticorps anti-PAS et d'un anticorps anti-phénacétine. L'action de la trypsine n'altère pas le site cellulaire, contrairement au tannin et au formol. Le site cellulaire est spécifique de l'espèce humaine.

dispensables à la réaction. Le mieux étudié a été le PAS (tableau 1) pour lequel la présence du radical COONa en position 1 et d'au moins un radical en position 2 ou 4 est indispensable [2]. On a même pu voir [3] que selon le malade les ractions croisées sont plus ou moins étendues (tableau 2), sans que ceci ne semble être lié au titre de l'anticorps.

Le support cellulaire de l'allergène.

Le point de fixation de l'allergène ou du complexe antigène-anticorps sur la cellule sanguine est intéressant à considérer.

Pour un même allergène le point de fixation pourra être selon les malades l'hématie, le thrombocyte ou le leucocyte ou même les tissus.

Des études très partielles ont été faites sur la nature de la substance réactive à la surface des hématies. On sait seulement que le traitement des hématies par la trypsine n'empêche pas la réaction. Il est donc

possible que la substance réactive ne soit pas protéinique. Par contre le traitement par le tanin ou le formol altère la réactivité.

La spécificité d'espèce a été trouvée limitée à l'espèce humaine dans les rares cas (PAS [2] et Phénacétine [4]) ou elle a été recherchée (tableau 3).

Les anticorps.

Les anticorps formés sous l'influence de ces produits sont hétérogènes. Ils peuvent varier

— dans leur spécificité,

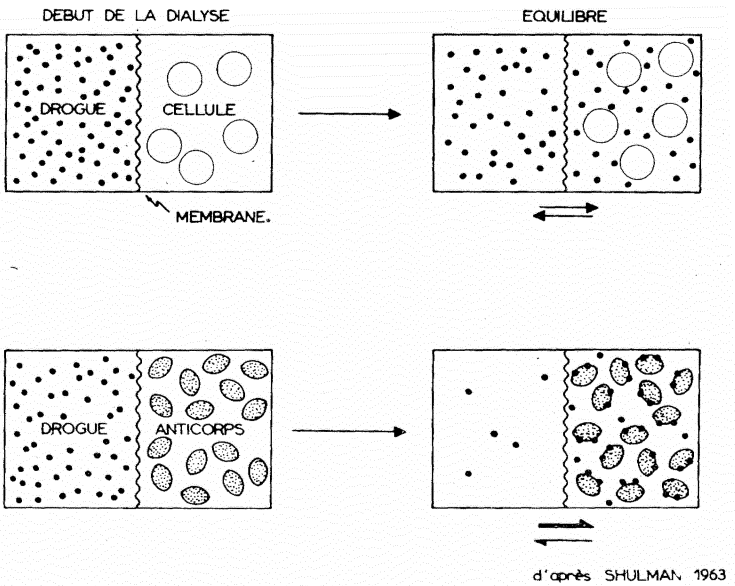

Tableau 4.—Expérience de SHULMAN (8) montrant la formation des complexes solubles en l'absence des cellules. Quand il y a un anticorps correspondant au médicament celui-ci est attiré vers le compartiment contenant l'anticorps.

— dans leurs caractères sérologiques,

— dans leurs caractères physico-chimiques.

En ce qui concerne leur spécificité nous avons vu qu'elle pouvait varier d'un malade à l'autre entraînant des réactions croisées plus ou moins étendues avec des produits voisins.

Les caractères sérologiques de ces anticorps sont ceux d'anticorps immuns, ayant leur optimum thermique à 37°. Ils sont probablement bi-valents puisqu'ils sont capables de provoquer l'agglutination. Des études récents [5] ont montré que la majorité d'entre eux sont des anticorps fixant le complément. L'intervention du complément dans la réaction est grande tant pour leur mécanisme d'action que pour leur détection.

NATURE DES ANTICORPS

MEDICAMENT	AGGLUTINANT				HEMOLYSANT	REAGISSANT
	en saline	PAR L'ANTIGLOBULINE AHC non-γ	AHG γ	Hématies trypsinisées	hématies normales ou trypsinisées ou PNH	directement avec le médicament
PAS	+	-	-	+	-	-
Phénacétine - Quinidine PAS	+	+	-	+	+	-
Quinine, Antistine Phénacétine, Salazopyrine, Chlorpromazine	-	+	-	-	(+)	-
Fuadine	+	+	+	+	+	-
Pénicilline Heptachlor	+	+	+		+	+
Pyramidon	-	-	-	+ seulement après traitement par pyramidon.	+	-

Tableau 5.—Etude sérologique des anticorps allergiques anti-hématies. Différents types d'anticorps (parfois développés contre le même médicament) sont observés. Les anticorps agglutinants non hémolysants sont l'exception. D'autres sont à la fois agglutinants, hémolysants et fixant le C'. La plupart sont hémolysants et fixant le C', décelés par l'agglutination par l'antiglobuline non-γ. Enfin pour certains la réaction directe avec le médicament est prouvée par les tests de neutralisation (3è mécanisme).

L'action lytique des anticorps allergiques est fonction de la fixation sur la cellule du C' et singulièrement du composant C'_3. Cette fixation était connue depuis les travaux d'ACKROYD [6]. Mais SHULMAN [7] en a précisé certains aspects. Il a montré que les composant β_1C (C'_3) et β_1E (C'_4) du complément humain se fixent à la cellule et que cette liaison était très ferme puisqu'elle persiste même après que l'anticorps et la drogue aient été éliminés.

Ce fait a une conséquence pratique importante car même après élimination du médicament par l'organisme, la trace de la fixation temporaire du complexe antigène-anticorps sur la cellule peut être retrouvée, grâce aux techniques de mise en évidence du complément en particulier par l'antiglobuline non-gamma.

Les méthodes de détection les plus sensibles sont celles qui font intervenir le complément

— pour les hématies, l'hémolyse ou le test de Coombs avec une antiglobuline non-gamma (tableau 5),

— pour les plaquettes, le test de déviation du complément, lyse ou inhibition de la rétraction du caillot,

— pour les leucocytes il n'a pas encore été démontré qu'une technique faisant intervenir le complément soit la plus sensible.

Il est cependant à côté de ces anticorps lytiques des anticorps allergiques ne fixant pas le C' et qui se sont montrés la cause d'accidents cytopéniques très graves. Ils peuvent être mis en évidence par agglutination, consommation de l'antiglobuline, etc...

Leurs caractères physico-chimiques sont variables. Ils sont constamment dans la fraction gamma-globulinique du sérum mais peuvent avoir une constante de sédimentation de S7 ou de S19. SHULMAN et RALL [8] ont observé que deux anticorps anti-stibophène actifs contre des érythrocytes étaient des S 19 alors que 6 anticorps anti-quinine ou quinidine mais actifs cette fois contre les plaquettes étaient des S7. Chez un malade possédant des anticorps anti-quinidine il a trouvé que les globulines S 19 réagissaient avec les hématies et les S7 avec les plaquettes. Il propose l'hypothèse que la nature physico-chimique de l'anticorps prédispose à l'atteinte de telle ou telle lignée cellulaire.

Les mécanismes.

De nombreuses discussions ont déjà été menées à ce propos. Le désaccord porte essentiellement sur le point de savoir si la *cellule* participe *activement* à la constitution même de l'antigène formant avec le produit médicamenteux un complexe qui devient antigénique (1er mécanisme) ou si la cellule n'est atteinte que *passivement* par un conflit immunologique qui se déroule à sa surface sans pour autant y participer (2è et 3è mécanisme). Mais une observation particulière laisse à penser qu'exceptionnellement un mécanisme tout à fait différent pourrait être en cause (4è mécanisme).

Enfin l'action des produits médicamenteux ne se limite peut-être pas à provoquer des anticorps du type allergique actifs seulement en présence du médicament. Il est possible que dans un plus grand nombre de cas qu'on ne le pense, ils puissent être à l'origine d'auto-anticorps.

1er mécanisme.

L'antigène est formé d'un complexe cellule-médicament (tableau 6). Ce mécanisme a été proposé por ACKROYD [6] sur la base de la négativité apparente de la réaction entre l'anticorps et la drogue, en l'absence des cellules. Par contre, dès que les trois éléments sont mis au contact on observe l'apparition d'un phénomène: l'agglutination, la lyse ou la fixation du complément.

Mais certains faits ont jeté un doute sur la réalité de ce mécanisme:

a) L'existence dans certains cas rares d'une réaction dès le mélange du sérum pathologique et de la drogue. Il peut se former dans certains cas un précipité.

b) La labilité extrême de la fixation de l'anticorps sur le complexe antigénique cellule-médicament. Cette labilité de la liaison antigène-

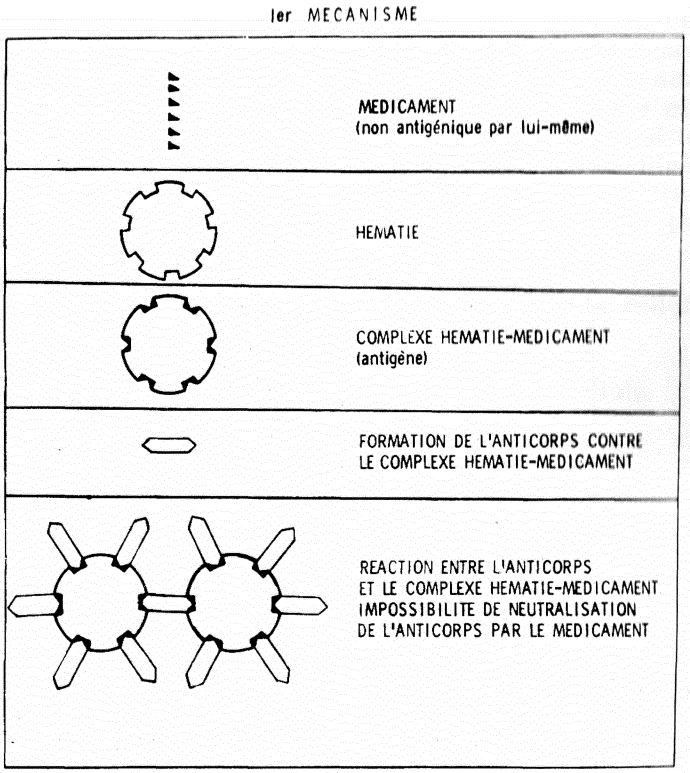

Tableau 6.—Premier mécanisme: l'antigène est constitué par le complexe cellule-médicament. La neutralisation de l'anticorps par le médicament est impossible, même à très forte concentration.

anticorps est inhabituelle en immunologie. Elle est montrée par la facilité avec laquelle par exemple par dialyse on peut dissocier l'anticorps et l'allergène de la cellule.

Quoiqu'il en soit ce mécanisme, bien que très séduisant, n'a pas été démontré expérimentalement.

2è mécanisme.

L'antigène est le médicament lui-même ou le médicament lié à une substance plasmatique (tableau 7). Des complexes solubles antigènes-anticorps se forment dans le plasma du malade et viennent *secondairement* se fixer sur les cellules.

Cette hypothèse formulée par MIESCHER [9] peut être assimilée à une immuno-adhérence d'un type spécial. Elle a été vulgarisée par SHULMAN [8] qui compare la cellule à un "passant innocent" sur lequel vient se fixer le complexe puis le complément. C'est ce dernier qui serait dans bien des cas l'agent destructeur.

A l'appui de cette hypothèse vient essentiellement une expérience de SHULMAN [7] qui montre que certaines drogues se fixent sur l'anticorps en l'absence de cellule. Si une solution de médicament est séparé du sérum contenant l'anticorps par une membrane de dialyse on voit après un certain temps qu'il y a plus de médicament dans le compartiment contenant le sérum que dans l'autre (tableau 4).

La réalité d'une réaction antigène-anticorps en l'absence des élé-

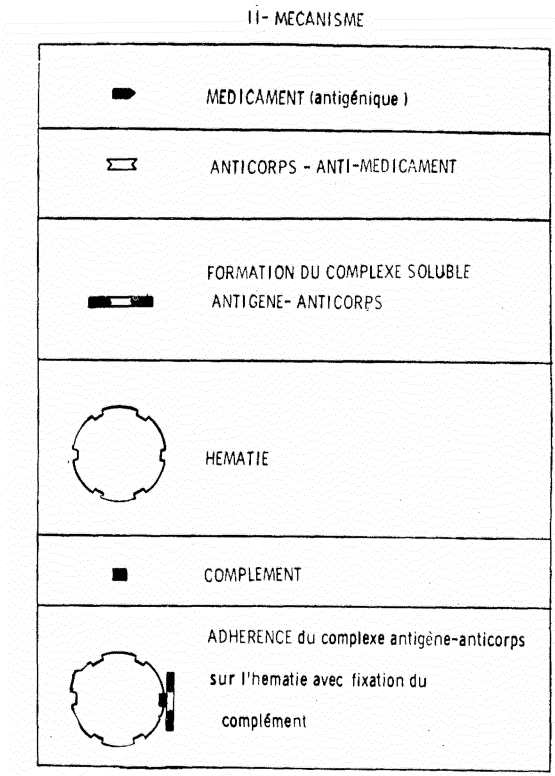

Tableau 7.—Deuxième mécanisme: l'antigène est le médicament lui-même (ou le médicoment lié à une substance plasmatique). Ce sont les complexes solubles Antigène/Anticorps qui viennent se fixer à la cellule, entraînant (éventuellement) la fixation secondaire du complément à la cellule.

ments figurés est donc prouvée. Cependant on ne compred pas encore pourquoi contrairement à ce qui est observé dans l'immuno-adhérence classique le complément n'est consommé que lors de la fixation des complexes sur la cellule et au moment de la formation de ces complexes.

3è mécanisme.

L'antigène est le médicament. C'est le complexe antigène-anticorps préalablement formé qui se fixe à la cellule mais la liaison se forme in

situ, à la surface même de la cellule entraînant la fixation du complément, d'où la lyse de la cellule.

Cette hypothèse voisine mais cependant différente de la précédente a été formulée par DAUSSET et CONTU [10] grâce à l'analyse d'observations privilégiées faites dans les anémies hémolytiques dues à la pénicilline [11] et à l'heptachlor [12].

Ces deux produits ont l'avantage sur les autres produits étudiés jusqu'ici de se fixer d'une façon ferme aux hématies rendant plus aisée l'étude du mécanisme.

Grâce à cette propriété il a pu être montré que:

a) Si on mélange en même temps les trois éléments, cellule, médicament, anticorps, la réaction se produit.

b) Si on procède en deux temps:

— 1er temps de fixation du médicament sur la cellule.

— 2è temps d'adjonction de l'anticorps,

la réaction se produit encore.

c) Si on procède également en deux temps:

— 1er temps de fixation du médicament sur la cellule;

— 2è temps d'adjonction du mélange médicament-anticorps, aucune réaction n'apparaît: elle est neutralisée.

Ces faits:

a) Apportent une confirmation de l'existence des complexes solubles et sont donc un argument à l'encontre du 1er mécanisme.

b) Suggèrent que l'antigène est le produit ou le médicament lui-même puisqu'il est capable à lui seul de neutraliser l'anticorps.

c) Suggèrent que le point d'attache du complexe antigène-anticorps sur le cellule est constitué par la liaison du médicament à la cellule puisque lorsque ces sites sont déjà occupés par le médicament la réaction ne peut plus se produire (à condition probablement que la réaction ait lieu en excès d'antigène).

Rien ne s'oppose dans l'état actuel de nos connaissances à ce que ce mécanisme démontré seulement pour ces quelques cas privilégiés s'applique également aux autres cas.

On sait que la pénicilline et l'heptachlor se différencient des autres produits allergisants par leur fixation ferme à la cellule.

Les sites occupés d'une façon ferme ne peuvent être libérés pour que les molécules de médicament qui sont liés aux anticorps viennent se fixer aux cellules. Par contre quand les sites ne sont occupés que d'une façon labile ils sont libérés et les molécules de médicaments engagés dans un complexe Antigène-Anticorps viennent s'y fixer et provoquent la réaction. La seule différence avec les autres médicaments résiderait dans la labilité de la fixation du médicament à la cellule mais le mécanisme intime pourrait rester la même.

Cette hypothèse apporte une meilleure compréhension des phénomènes d'agglutination. Quoique les complexes solubles adhérés à des cellules soient connus pour être capables de provoquer l'agglutination, il est plus aisé de concevoir celle-ci comme étant la conséquence d'une liaison entre deux cellules par un anticorps bivalent.

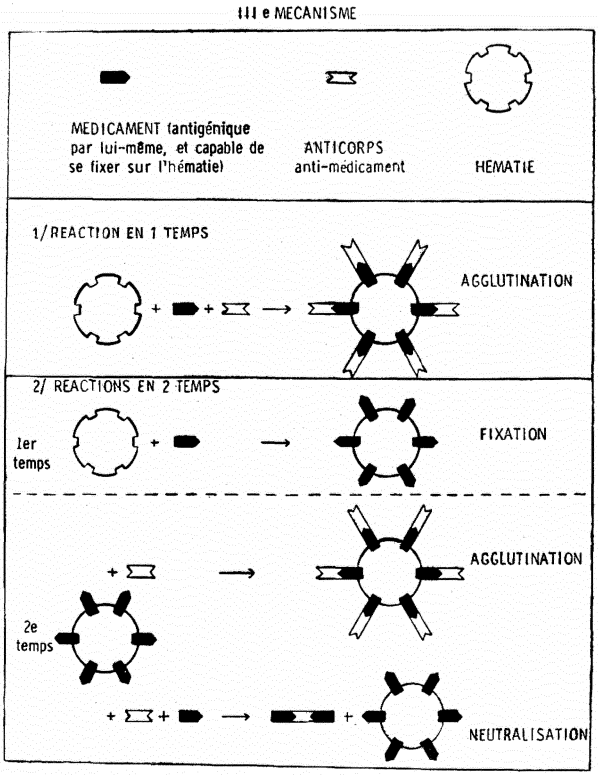

Tableau 8.—Troixième mécanisme: l'antigène est le médicament lui-même. Il adhère à le cellule. L'anticorps se fixe sur lui, entraînant (éventuellement) la fixation secondaire du complément à la cellule.

Le fait que le complément ne se fixerait pas ou faiblement sur les complexes solubles et fortement sur les cellules porteuses du complexe antigène-anticorps doit être enregistré. Il n'est pas mieux expliqué par cette hypothèse que par la précédente.

Cette hypothèse suppose donc que le médicament est l'antigène même et que la liaison avec la cellule est une liaison espécifique entre le médicament et la surface de la cellule. Elle ne fait pas comme l'hypothèse précédente intervenir directement les complexes solubles bien que ceux-ci existent et ne fait pas d'analogie avec l'immuno-adhérence.

Elle a l'avantage sur les autres hypothèses de reposer sur des faits expérimentaux classiques. On connaît l'*hémolyse passive in vitro* obtenue chez l'animal par INGRAHAM [13] et par SILVESTEIN et MALTANER [14].

Il s'agirait donc, pour la série érythrocytaire d'une *hémolyse passive in vivo* et pour les autres séries d'une *lyse passive in vivo*.

4ème mécanisme.

L'antigène est la cellule altérée par le médicament (tableau 9). Un cas très particulier et unique décrit par BERNASCONI et al [15] d'anémie hé-

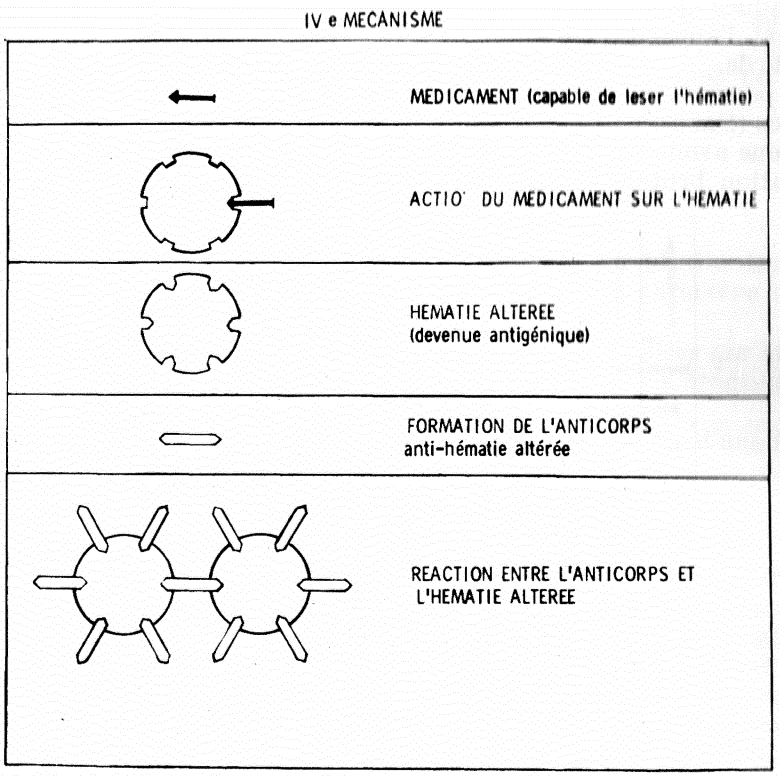

Tableau 9.—Quatrième mécanisme: l'antigène est la cellule modifiée par le médicament. L'anticorps se fixe sur l'antigène cellulaire modifié (la fixation du complément n'a pas été observé dans le cas unique (15) où ce mécanisme est le jeu).

molytique au pyramidon nécessite une 4ème hypothèse. En effet, le mélange du sérum malade, du médicament et des hématies (même trypsinisées) n'entraîne pas l'agglutination, ce qui exclut les deux premiers mécanismes. L'anticorps n'est pas neutralisé par le médicament. Par contre, le sérum malade est capable d'agglutiner des hématies normales ayant subi un contact prolongé avec le médicament puis trypsinisées. Tout se passe comme *si le médicament altérait l'hématie, la rendant antigénique par elle-même.* La trypsine n'apparaît nécessaire que pour révéler cet antigène modifié.

Déclenchement de la formation d'auto-anticorps.

Le 4ème mécanisme permet de faire le pont entre les cytopénies allergiques de caractère brusque, dramatique mais rapidement récessif dès l'arrêt du médicament et certaines cytopénies médicamenteuses plus lentement récessives voire chroniques.

On sait que l'altération tissulaire par exemple des fibres musculaires

cardiaques après cardiotomie ou après infractus du myocarde entraîne souvent l'apparition d'auto-anticorps spécifiquement dirigés contre le myocarde.

Il pourrait en être de même pour les leucocytes, voire pour les plaquettes (DAUSSET [16]).

Nous avons en effet observé quelques cas très caractéristiques d'intoxication brutale par des produits réputés cytopéniants (tableau 10)

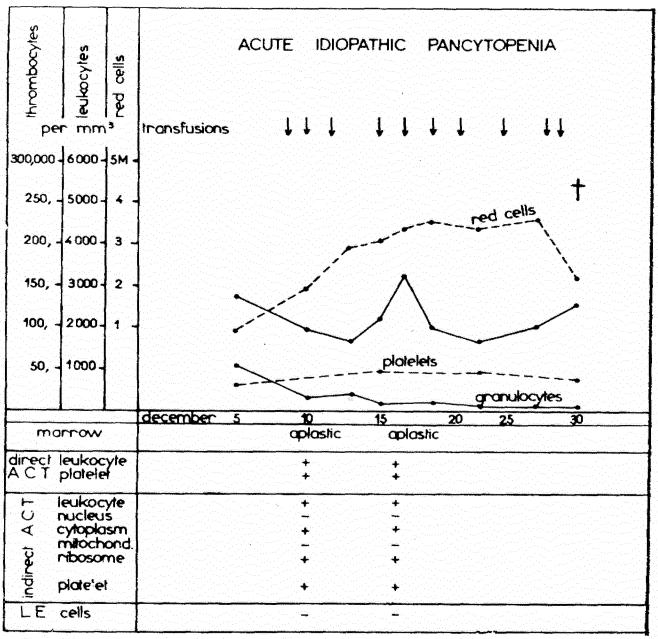

Tableau 10.—Exemple d'agranulocytose brutale s'accompagnant d'auto-anticorps anti-leucocytes décelés par la consommation directe (sur les propres leucocytes du malade) et indirecte (sur les leucocytes étrangers) de l'antiglobuline.

qui s'accompagnaient d'agranulocytoses profondes et chez lesquels on pourrait déceler par les tests directs et indirects de consommation d'antiglobuline sur les leucocytes une substance gamma globulinique ayant les caractères sérologiques d'auto-anticorps anti-leucocytaires. Chez ces malades la guérison a été complète et les tests sont revenus à la normale.

Nous nous sommes demandé de ces leucopénies chroniques ne pouvaient relever d'un mécanisme analogue. En effet ces leucopénies chroniques dîtes idiopathiques frappent le plus souvent des malades au passé pathologique chargé, ayant entraîné un long usage médicamenteux (névropathies, rhumatismes). Chez eux également on note des tests positifs de consommation de l'antiglobuline sur leucocytes, voire sur leucocytes et sur plaquettes quand les deux lignées sont atteintes. Il faudrait supposer dans ce cas l'existence d'un auto-anticorps permanent, perpétuant la cytopénie.

Mais ces accidents chroniques peuvent néanmoins relever du mécanisme d'allergie médicamenteuse classique. Des purpuras thrombopéni-

ques d'allure chronique ont été confondus pendant de longs mois avec des purpuras thrombopéniques chroniques idiopathiques et se sont révélés être en fait d'origine allergique, entretenus par des prises fréquentes, d'acide acétyl-salicylique contre lequel s'était développé un anticorps [17].

Caractère individuel à la réponse allergique.

A la complexité du mécanisme vient s'ajouter la complexité des conditions d'apparition et la difficulté de comprendre le caractère apparemment *individuel* des sensibilisations.

Pour souligner ce caractère individuel il suffit de rappeler que sur un nombre parfois considérable d'individus soumis à certaines drogues (par exemple la quinine) seuls certains présentent des accidents.

Bien mieux, l'immunisation à un même médicament varie d'une façon très remarquable d'un individu à l'autre. Ces variations peuvent porter :

1.° Sur le type sérologique d'anticorps.

2.° Sur sa spécificité.

En effet, un même médicament pourra provoquer tantôt l'apparition d'anticorps de type allergique cytopéniant. L'exemple le plus caractéristique en est fourni par le pyramidon. Le malade étudié par HALPERN [18] possédait un puissant anticorps de type réaginique et l'accident ne s'accompagnait d'aucune leucopénie. Il ne possédait pas d'anticorps, agglutinant les leucocytes en présence de pyramidon. A l'inverse la malade que nous avons étudiée [19] possédait de tels anticorps mais aucun anticorps de type réaginique.

La spécificité des anticorps allergiques cytopéniants peut également varier d'un malade à l'autre, atteignant d'une ou l'autre des lignées cellulaires du sang. Tel produit entraînera selon le malade une anémie hémolytique, un purpura thrombopénique ou une leucopénie. La majorité des produits ayant provoqué des anémies hémolytiques allergiques ont provoqué chez d'autres malades des anticorps actifs tantôt sur les leucocytes tantôt sur les plaquettes [10], alors qu'il n'en est pas ainsi d'un anticorps donné dont la spécifité reste le plus souvent confinée à une seule lignée.

Enfin, la spécificité des anticorps provoqués par un même médicament peut varier pour une même lignée cellulaires selon les corps chimiquement voisins utilisés. Ainsi la comparaison de plusieurs anticorps allergiques anti-PAS étudiés par agglutination érythrocytaire montre des différences de spécificité importantes quand on étudie une gamme de corps voisins au PAS. Ces différences ne semblent pas pouvoir s'expliquer par des différences de titre. La configuration même de l'anticorps est apparemment différente [3].

On est tenté dès lors de penser à des caractères héréditaires constitutionnels qui favoriseraient le développement des anticorps allergiques. Mais aucun argument ne vient chez l'homme étayer cette hypothèse :

parmi les familles soumises en même temps à la quinine aucun cas de purpura thrombopénique familial n'a été signalé! De même il n'apparait pas que les accidents hémolytiques de type allergique soient plus fréquents dans les populations où les tares enzymatiques érythrocitaires sont les plus répandues; pas plus fréquents non plus chez les atopiques. Mais avant d'écarter la notion de pharmaco-génétique faudrait-il avoir rechercher dans les familles des malades des sensibilisations latentes, dans le cas de contact familial avec un produit. Nous avons pu observer en effet des sensibilisations latentes découvertes par des examens fortuits [20].

Enfin le rôle de l'hérédité peut être masqué s'il s'agit d'un caractère récessif nécessitant la conjonction chez un même individu de deux caractères rares. Chez l'animal une étude récente[21] montre que ce sont les mêmes animaux qui sont capables de se sensibiliser contre plusieurs haptènes conjugués par diazotation avec des proteines, indiquant que ces animaux métabolisent ces conjugués hapténiques d' une certaine manière les rendant antigéniques. Cette observation renforce notre penchant à admettre des différences individuelles constitutionnelles à la base du caractère facultatif des accidents.

Le caractère individuel pourrait d'ailleurs porter sur les différents stades du métabolisme:

1.° Sur le catabolisme même du medicament.

2.° Sur la constitution du support cellulaire sur lequel le médicament ou son métabolite pourra se fixer.

3.° Enfin sur la réponse du systéme immunitaire. Tout nous est encore inconnu dans cette chaîne de réaction.

Pathogénie.

Enfin on est en droit de soulever un problème très important, celui du rôle pathogénique de ces anticorps.

Si dans la plupart des cas le rapport entre la présence de ces anticorps et l'accident cytopéniant est évident, dans d'autres cas on est frappé par l'absence de cytopénie malgré la présence d'anticorps apparemment identiques à ceux que l'on rencontre chez les malades ayant présenté une cytopénie. On trouve dans la littérature des anticorps anti-quinine, sans anémie ou thrombopénie; des anticorps anti-PAS entraînant des chocs analogues à des chocs transfusionnels mais sans cytopénie et enfin de très nombreux anticorps anti-pénicilline. Cependant dans ce dernier cas on a signalé que des hématies marquées et probablement imprégnées de pénicilline étaient éliminées rapidement.

Il est probable que de nombreux facteurs génétiques ou occasionnels interviennent pour rendre les anticorps pathogènes ou inoffensifs. Parmi les facteurs occasionnels citons le rapport allergène/anticorps, le rapport allergène/support cellulaire. Ces deux rapports peuvent varier considérablement et ce n'est probablement que lorsque, l'un et l'autre de ces rapports se situent dans des valeurs relativement précises que l'accident peut survenir.

En conclusion des hématies d'origine allergique commencent à être mieux connues. Un grand nombre de produits médicamenteux ont été montré être responsables de ces accidents cytopéniques. Ce sont essentiellement les plaquettes qui sont atteintes, mais les hématies, voire les leucocytes, ne sont pas épargnées. L'accident porte généralement sur une seule lignée mais peut dans certains cas rares, porter sur deux des lignées cellulaires du sang. Un même produit peut, selon les malades, entraîner une anémie, un thrombopénie, ou une leucopénie. Il peut ainsi provoquer un choc, ou des réactions cutanées du type allergique sans cytopénie. Il peut enfin être parfaitement toléré même en présence du médicament responsable.

Plusieurs problèmes restent sans réponse. Le mécanisme de ces accidents brutaux est discuté. Pour les uns le médicament ne serait pas antigénique par lui-même mais le deviendrait après liaison avec une substance à la surface de la cellule. Pour d'autres le médicament constituerait l'antigène et c'est le complexe antigène-anticorps ou même le médicament lui-même qui irait se fixer sur la cellule. Celle-ci comparée à un passant innocent, soufrirait passivement du conflit immunologique qu'elle supporte. Dans un cas unique l'antigène paraît être l'hématie altérée par le médicament.

Le mystère du caractère facultatif pour ainsi dire individuel de ces accidents demeure. Il n'y a pas de notion familiale, de race ou de sexe. La nature même de l'anticorps et sa spécificité dépend de l'individu qui l'a fabriqué. Il est probable que de nombreux facteurs génétiques et occasionnels interviennent pour rendre ces anticorps pathogènes et pour leur donner leur spécificité.

Enfin à côté des anticorps allergiques désormais classiques, il pourrait exister des auto-anticorps dirigés contre les cellules sanguines et développés à la faveur d'une altération des antigènes cellulaires par un produit médicamenteux. Ils seraient la conséquence puis peut-être la cause de la cytopénie prolongée. Cette hypothèse est soulevée par analogie aux auto-anticorps que l'on peut observer après certains délabrements similaires.

De nombreuses études sont encore nésessaires pour élucider la complexicité des mécanismes allergiques capables d'entraîner des hémopathies mais la place de ces anticorps en hématologie ne cesse de s'étendre au fur et à mesure que la liste des produits s'allonge et que les techniques de détection de ces anticorps se perfectionnent.

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LEBERERKRANKUNGEN UND ALLERGIE

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Im Rahmen des übergeordneten Themas "Auto-Sensibilisierung" sollen bezüglich der Lebererkrankungen 3 grundsätzliche Fragen zur Diskussion gestellt werden:

I. Können Lebererkrankungen primär-allergisch bedingt sein?

II. Was wissen wir über immunologische Phänomene bei primär *nicht* allergischen Lebererkrankungen?

III. Welche Bedeutung haben diese Phänomene für die klinische Diagnostik, für das Schicksal der jeweiligen Lebererkrankung, für Krankheitskomplikationen und für die entzündliche Beteiligung der Leber im Rahmen extrahepatischer Primärerkrankungen?

I. ALLERGISCH BEDINGTE LEBERERKRANKUNGEN.

Es gibt Beobachtungen, die darauf hindeuten, dass die Leber wie die Gallenwege Erfolgsorgan von Überempfindlichkeitsreaktionen gegenüber exogenen Fremdantigenwirkungen sein können. *Unter klinischen Bedingungen* kennen wir eine entzündliche Leberbeteiligung mit Ikterus bei der Serumkrankheit. Sie hat ihr Pendant im Nachweis entzündlicher Lebergewebsveränderungen nach experimentell erzeugtem anaphylaktischen Schock. Klinisch pflegt der Ikterus in diesen Fällen in direktem zeitlichem Zusammenhang mit anderen Erscheinungen der Serumkrankheit (Gelenkbeteiligung, Exanthem, Lymphadenopathie) aufzutreten, also ohne ein weiteres Zeitintervall, wie man dies etwa bei der infektiösen Hepatitis nach den ersten Prodromalerscheinungen erwarten würde (CULLINAN). Von KAEGER ist sogar der Fall einer akuten Lebernekrose beschrieben, der im Gefolge einer Serumkrankheit nach Verabfolgung eines Tetanus-Antiserum vom Pferd zur Ausprägung kam.

Entzündliche Lebererkrankungen können aber auch nach Transfusionszwischenfällen bei Rh-Unverträglichkeit auftreten. Eosinophile Zellinfiltrate und seröse, interstitielle Exsudatbildungen in der Leber sind das morphologische Substrat.

Klinisch wichtiger sind Leberschädigungen infolge Arzneimitteleinwirkungen, die nicht nur toxisch, sondern auch allergisch bedingt sein können. Sie treten klinisch in 2 Formen, nämlich als *hepatozellulärer Ikterus* mit Leberzellnekrosen—oder aber als Gelbsucht mit Verschlusssyndromen infolge *allergischer Cholangiolitis* in Erscheinung (POPPER und SCHAFFNER; G. A. MARTINI).

Die ersterwähnte Form kann durch Überempfindlichkeit gegenüber Sulfonamiden, Antibioticis oder Atophan ausgelöst sein, ist in ihrer allergischen Prägung aber sicher selten und häufiger toxisch bedingt, geht dann zumeist allerdings *ohne* Ikterus einher. Bei den allergischen Formen finden sich herdförmige Nekrosen in der Leber, die von plasmazellulären Infiltrationen begleitet sind. In diesen Fällen mit Ikterus kann die Differentialdiagnose zur Virushepatitis sowohl nach klinischen wie nach morphologischen Kriterien (Leberpunktion) ausserordentlich schwierig sein. Die Ähnlichkeit hat sogar daran denken lassen, nicht die medikamentöse Allergie als solche, sondern eine durch sie bedingte Manifestation einer bis dahin latenten Virushepatitisinfektion sei die Grundlage des klinischen Geschehens.

Nach POPPER steigt die zweiterwähnte Form der *allergischen Cholangiolitis* infolge Überempfindlichkeitsreaktionen gegenüber Medikamenten in ihrer Häufigkeit ständig an. Histologisch ist diese Form durch zentralobuläre Stase mit pericholangiolären Infiltraten gekennzeichnet, wobei eosinophile Zellen beteiligt sind. Klinisch finden sich Ikterus mit Juckreiz, Stuhlentfärbung, Leber- und Milzschwellung, starke Erhöhung der alkalischen Phosphatase wie des Gesamtcholesterins im Serum, aber weitgehend normale Labilitätsteste. Auch die Transaminasen werden nur mässig erhöht gefunden; eine Bluteosinophilie gilt als charakteristisch. Die klinische Abgrenzung zum mechanischen Verschlussikterus ist darum so wichtig, weil Patienten mit allergisch bedingter Cholostase fehlindizierte operative Eingriffe besonders schlecht vertragen. Zahlreiche Medikamente, unter ihnen Chlorpromazin (Megaphen), Favistan, Thiouracil, wiederum auch Sulfonamide und Antibiotica können dieses Bild der intrahepatischen Cholostase auslösen. Nicht selten dürfte es sich aber auch hier um toxische Einflüsse handeln, nicht durchweg um echte allergische Reaktionen.

Verallgemeinert, gewinnt man damit den Eindruck, dass primärallergische Leberschädigungen in den beiden genannten klinischen Formen zwar möglich sind, doch seltener als vielfach angenommen.

II. IMMUNOLOGISCHE PHÄNOMENE BEI LEBERERKRANKUNGEN PRIMÄR NICHT ALLERGISCHER GENESE.

Auch bei solchen Lebererkrankungen meist infektiöser oder entzündlicher, vielleicht auch toxischer Genese, die also sicher nicht primärallergisch entstanden sind, kennen wir zahlreiche immunologische Phänomene von unterschiedlicher klinischer Bedeutung.

a) *Auto-Antikörper gegen Lebergewebe:*

Bereits im Jahre 1907 bzw. 1908 haben CENTANNI wie unabhängig von ihm FIESSINGER das Auftreten präzipitierender bzw. complementfixierender Antikörper gegen Lebergewebe bei der menschlichen Lebercirrhose beschrieben. Die Ergebnisse blieben später aber Jahrzehnte hin unbeachtet, bis 1944 EATON und Mitarb. über das Auftreten eines complementfixierenden Antikörpers im Verlauf der Virushepatitis berichtet haben, der unter Verwendung von Extrakten aus Hepatitis Lebern, aber auch aus gesunden menschlichen Lebern zur Darstellung gebracht werden konnte.

Wir selbst haben zuerst 1953 über entsprechende Antikörpernachweise bei insgesamt 105 Fällen ätiologisch verschiedenartigster Lebererkrankungen berichtet. Die untersuchungen wurden mit der Complementbindungsreaktion und mit der passiven Hämagglutination durchgeführt, als Antigene fanden Kochsalzextrakte aus gesundem menschlichen Lebergeweve Verwendung, wobei unspezifische Begleitproteine durch vorhergehende Fällungsprozesse ausgeschaltet wurden. Positive Antikörpernachweise erwiesen sich ausschliesslich auf die Virushepatitis und ihre Folgezustände, hier vor allem auf die Übergangsformen zur chronischen Krankheitsentwicklung beschränkt (72 von 105 Fällen). Durchweg positive Befunde wurden bei biliärer Lebercirrhose (13 Fälle) ermittelt. Zum Vergleich untersuchte Erkrankungen an mechanischem Verschlussikterus, an Lebertumoren, Miliartuberkulose der Leber und Stauungsleber (20 Fälle) wurden durchweg serologisch negativ gefunden. Analoge Ergebnisse haben wir später im Agar-Gel-Diffusionstest ermittelt und damit präzipitierende Antikörper gegen Lebergewebe sichtbar gemacht. In allen diesen Untersuchungen ergab sich, dass die Frühstadien der Virushepatitis negativ bleiben, so dass der positive Antikörperbefund nur den Spätstadien der Virushepatitis, vor allem aber den Übergangsformen zur Chronizität zukommt. Parallelen zur Bewegung der Transaminasen wurden nicht ermittelt, der hohe Anstieg der Fermente im Serum erfolgte sehr viel früher als das Positivwerden der Antikörperbefunde.

An der Gesetzmässigkeit dieser Antikörperbefunde bei entzündlichen Lebererkrankungen dürfte heute kein Zweifel mehr sein: 1958 teilten GAYDUSSEC u. Mitarb. Nachprüfungen mit gleichlautendem Ergebnis in der Complementfixation mit. 1960 wiesen HUNTER u. Mitarb. zirkulierende Auto-Agglutinine gegen Lebergewebe vor allem bei den chronisch fortschreitenden Krankheitsentwicklungen nach, 1961 kamen ASHERSON und PROBERGER zu gleichlautenden Befunden, wobei von diesen Autoren Phenol-Wasser-Extrakte aus menschlichem Lebergewebe verwendet wurden. 1962 fanden PASNIK u. Mitarb. die Complementfixation mit Leberextrakten bei 874 Untersuchungen ganz überwiegend den chronisch progredienten Hepatitisformen zugeordnet, dann aber auch bei Leberbeteiligungen im Rahmen eines Lupus erythematodes visceralis positiv. ASHERSON und PROBERGER haben 1961 ebenfalls bereits eine

Krankheitsspezifität dieser Befunde abgelehnt, denn auch sie hatten positive Resultate bei Lupus erythematodes visceralis mit Leberbeteiligung, dann auch bei Miterkrankung der Leber im Rahmen des entzündlichen Rheumatismus. MACKAY kam 1960 zum Nachweis positiver Antikörperbefunde ebenfalls bei der biliären Lebercirrhose; prinzipiell gleichlautende Befunde sind durch DAUSSET, durch STEFFEN, durch SCHEIFFAHRT und Mitarb. erhoben worden, um nur die wichtigsten Untersucher zu nennen.

POPPER und Mitarb. haben als erste die Fixation von Gamma-Globulin-Antikörpern im menschlichen Lebergewebe mit fluoreszenzserologischen Methoden sichtbar gemacht und fanden dabei zwei verschiedene Reaktionstypen: sie wiesen nach, dass nicht selten Gamma-Globulin-Antikörper an Kernsubstanzen gebunden werden, ähnlich den antinucleären Faktoren des Lupus erythematodes, doch ohne Auftreten eines L. E.-Zellphänomens. Als zweiten Reaktionstyp haben PARNETTO, SHAFFNER, POPPER u. Mitarb. wiederum mit Hilfe der Fluoreszenztechnik zirkulierende Antikörper aufgedeckt, die gegen das Cytoplasma von Gallengangszellen gerichtet sind. Um den Ort der Antikörper-Fixation genauer zu bestimmen, fanden 20 autoptisch gewonnene Lebern Verwendung, 10 mit dem Befund einer postnekrotischen Cirrhose oder einer primärbiliären Lebercirrhose, 10 andere Lebern mit dem Befund einer postalkoholischen Fettcirrhose. Nur bei der ersten Gruppe fand sich eine stärkere Proliferation der kleinen Gallengänge, nur hier ergab sich eine positive Fluoreszenz. Die Bindung des Antikörpers an das zelluläre Substrat erfolgte unter Complementverbrauch. Unter insgesamt 195 klinischen Fällen wurden antiductuläre Antikörper

in 75 % bei der biliären Lebercirrhose nachgewiesen,
in 67 % bei der Virushepatitis und ihren Folgezuständen und
in 47 % bei der postnekrotischen Lebercirrhose.

Der Grad der Antikörperfixation stand in Parallele zur Ausdehnung und Schwere der Gallengangsproliferation.

Das verantwortliche "Auto"-Antigen der Leber muss im Cytoplasma der Gallengangszellen gelegen sein. Chemisch kann es sich um einen Carbohydrat-Protein-Komplex handeln. Die durch Antikörperbindung ausgezeichneten Zellen zeigten durchweg eine Zunahme des basophilen Cytoplasmas und vermehrte PAS-positive Granula. Diese Zellen erwiesen sich auch durchweg von einer entzündlichen, mesenchymalen Reaktion umgeben.

Der verantwortliche Antikörper ist ein Gamma-Globulin und auch durch langfristige Cortisonbehandlung in seiner Bindung an das zelluläre Substrat unbeeinflussbar. Eine Korrelation der Antikörperbindung zu histologischen Aktivitätszeichen der Entzündung ergab sich nicht. Möglicherweise werden diese Antikörper auch bei Virushepatitis gebildet; kommt es zur Heilung, verschwindet das antigenwirksame Zellsubstrat relativ schnell, die zur Ausbildung gekommenen Antikörper entbehren dann das antigene Substrat, Reaktionen zwischen beiden bleiben aus. Mit chronisch entzündlicher Progredienz wird demgegen-

über das antigene Substrat in erheblichem Masse ausgebildet, die Antigen-Antikörper-Reaktion bleibt langfristig persistent.

b) *Weitere serologische Phänomene bei entzündlichen Lebererkrankungen*:

Es steht ausser Zweifel, dass unter bestimmten klinischen Umständen, auf die noch zurückzukommen sein wird, auch antinucleäre Serumfaktoren auftreten können, selbst wenn das klinische Bild eines visceralen Erythematodes *nicht* voll ausgeprägt ist. SCHENKER u. Mitarb. haben das transitorische Auftreten eines L. E.-Zellphänomens sogar bei einem Fall von Virushepatitis beschrieben. Dennoch ordnen sich die antinucleären Faktoren und auch das positive L. E.-Zellphänomen weder der Virushepatitis und ihren Folgezuständen noch den biliären Cirrhoseformen gesetzmässig zu. Sie werden vielmehr zum Symptom der besonderen Form einer chronisch progredienten, interstitiellen Hepatitis, deren klinische wie immunologische Sonderstellung heute vielfach unter dem Begriff der "lupoiden Hepatitis" zusammengefasst wird (vgl. dort).

Eigene Untersuchungen (ROSS und VORLAENDER) haben weiter gezeigt, dass bei visceralem Erythematodes mit Nieren- und Leberbeteiligung Antikörper gegen cytoplasmatische Antigene auftreten, die *nicht* organspezifisch sind, dennoch aber quantitativ in jenen Organen konzentriert werden, deren entzündliche Beteiligung klinisch im Vordergrund steht. WIEDERMANN, DOERMER und MIESCHER haben diese Befunde neuerdings bestätigt, fanden identische cytoplasmatische Antikörper aber auch bei reiner Virushepatitis und deren Folgezuständen, d. h. also auch ausserhalb des visceralen Erythematodes. Daraus wird deutlich, dass im Verlauf entzündlicher Lebererkrankungen mehrere und voneinander verschiedene cytoplasmatische Antikörper auftreten, die auch bezüglich ihrer möglichen pathogenen Wirkung voneinander unterschieden werden müssen. Schliesslich ist auch ihr Substrat im Interstitium der Leber nicht identisch, cytoplasmatische Antikörper der letzterwähnten Kategorie werden werden weder bevorzugt noch ausschliesslich in Gallengangszellen gefunden.

Mit diesen Befunden sog. Auto-Antikörper ist die Zahl klinisch wichtiger serologischer Phänomene bei entzündlichen Lebererkrankungen nicht erschöpft. Wir wissen, dass vor allem bei der biliären Lebercirrhose und bei der lupoiden Hepatitis auch die Rheuma-Faktoren in hohem Titer "falsch-positiv" in Erscheinung treten können. Dieser Befund ist wiederum durchweg der Tendenz zur entzündlichen Progredienz zugeordnet und daher ein diagnostisches Symptom von nicht zu unterschätzender klinischer Bedeutung. BOUCHIER u. Mitarb. haben erst in jüngster Zeit bestätigt, dass das Auftreten der Rheumafaktoren jedoch nicht ausschliesslich der biliären Lebercirrhose und der lupoiden Hepatitis vorbehalten bleibt, sondern dass man das gleiche Phänomen auch bei den postnekrotischen Cirrhosiformen beobachten kann. Allerdings sind die Titer hier durchweg erheblich niedriger als bei den erstgenannten Formen chronisch entzündlicher Lebererkrankungen.

Um die Übersicht zu erleichtern, sei schon an dieser Stelle erwähnt, dass man im Verlauf chronisch entzündlicher Lebererkrankungen nicht selten auch hohe Titer von Kälteagglutininen findet und dass darüberhinaus antierythrocytäre Wärmeantikörper wie auch antithrombocytäre Auto-Antikörper nachweisbar werden können, wenn immunhämatalogische Komplikationen bei entzündlichen Lebererkrankungen vorliegen.

c) *Die vergleichsweise Zuordnung dieser serologischen Befunde zur Dysproteinämie*:

Das Verständnis für die Vielzahl und Verschiedenartigkeit in der klinischen Bedeutung dieser Seroreaktionen bei entzündlichen Lebererkrankungen wird erleichtert durch die vergleichende Zuordnung zur Analyse des Serumeiweissbildes:

Bei der akuten Virushepatitis findet sich die Reaktionskonstellation der akuten bzw. chronischen Entzündung mit Albuminverminderung und relativer Gamma-Globulinvermehrung. Dabei geht der Grad der Albuminverminderung mit dem Schweregrad der akut entzündlichen Erscheinungen parallel (MÜTHING). Übergänge zur chronischen Krankheitsentwicklung zeigen eine zunehmende Vermehrung der Gamma-Globuline. Eine besonders frühzeitige und excessive Gamma-Globulinvermehrung verbindet sich nicht selten mit dem Übergang zur akuten Lebernekrose und besitzt somit diagnostischen Wert. Alle antinucleären Antikörper, aber auch die Mehrzahl anticytoplasmatischer Antikörper sind Gamma-Globuline. Daraus wird verständlich, dass die serologischen Phänomene um so deutlicher zur Ausprägung kommen müssen, je deutlicher sich mit chronisch entzündlicher Progredienz dieser Gamma-Dysproteinämietyp ausprägt. Mit Entwicklung zur atrophischen Cirrhose hin tritt andererseits eine Verminderung der Alpha$_2$-Globuline als zusätziliches diagnostisches Kriterium dieser Entwicklung in den Vordergrund.

Ganz anders liegen die Verhältnisse bei der *biliären Cirrhose,* wo sich zwar auch der Gamma-Dysproteinämietyp findet, hier jedoch verbunden mit einer Vermehrung der Alpha$_2$- und Beta-Globuline sowie mit einer deutlichen Vermehrung der Beta$_2$-Makro-Globuline. Diese Fraktion, von der wir heute wissen, dass sie ebenfalls Träger wichtigster Antikörperfunktionen ist, lässt sich nur immunelektrophoretisch analysieren.

Rheumafaktoren sind Beta$_2$-Makro-Globuline, von einer Sedimentationskonstante 19 S, die mit Gamma-Globulinen von der Sedimentationskonstante 7 S in Reaktion treten. Darauf beruhen die Nachweisverfahren im Waaler-Rose-Test und Latex-Gamma-Test (vgl. auch bei VORLAENDER). Auch Kälte-Antikörper und Auto-Häm-Antikörper sind zum Teil Beta$_2$-Makro-Globuline, das vermehrte Auftreten aller dieser Faktoren gerade bei den biliären Cirrhoseformen wird also leicht verständlich. Die Gesamtzunahme aller dieser Serumfraktionen führt zu einer Vermehrung des Gesamteiweisses im Blut, die so deutlich werden kann,

dass differentialdiagnostisch nicht selten eine Leberbeteiligung bei Plasmozytom oder Morbus WALDENSTROEM erwogen wird. Wiederum bringt die Immunelektrophorese den Beweis oder Ausschluss der Paraproteinbildung und damit die diagnostische Klärung. Der Ausschluss von Paraproteinen bei gleichzeitigem Nachweis einer Antikörperfixation im Lebergewebe beweist die isolierte, chronisch entzündliche Lebererkrankung.

III. DIE KLINISCHE IMMUNOLOGIE ENTZÜNDLICHER LEBERERKRANKUNGEN.

Wir kommen damit zu vorwiegend klinischen Gesichtspunkten zurück und zur Beantwortung der Fragen:

nach der klinisch diagnostischen Bedeutung dieser immunologischen Vorgänge,

nach ihrer Bedeutung für die Pathogenese von Krankheitskomplikationen,

nach der möglichen Miterkrankung der Leber im Rahmen extrahepatischer Primärerkrankungen und

schliesslich zur Beantwortung der Frage, ob Beweis dafür vorliegen, dass das immunologische Geschehen für die Entwicklungstendenz, also das Schicksal einer bestimmten Lebererkrankung, auch kausal verantwortlich sein kann.

a) *Klinisch-diagnostische Ergebnisse:*

Im Folgenden soll versucht werden, definierten Formen entzündlicher Lebererkrankungen das Verhalten der Serumeiweisskörper und die mögliche Zuordnung bestimmter immunologischer Phänomene tabellatisch gegenüberzustellen, um daraus unter Anlegung kritischer Massstäbe Rückschlüsse für die klinische Diagnostik zu ziehen.

Akute Hepatitis mit Heilungstendenz:

Klinische Daten: Prodromi mit Magen-Darm-Erscheinungen, evtl. mit Gelenk-Symptomen, Icterus, schmerzhafte Leberschwellung, Transaminasen-Vermehrung über 500-1.000 DROBLEWSKI-Einheiten, geringe Erhöhung der alkalischen Phosphatase, wenig pathologischer Ausfall der Labilitätstests, Erhöhung des Eisenwertes im Blut, Prothrombinverminderung.

Pathologisch-anatomische Kriterien: Fleckförmige Nekrosen, interstitielle Infiltrate mit Histiocyten, Lymphocyten, Leucocyten und Plasmazellen in den periportalen Feldern.

Chronisch-interstitielle Hepatitis mit Übergang in postnekrotische Lebercirrhose:

Klinische Daten: Chronische, anikterische oder subikterische Leberschwellung mit vermehrter Urobilinogenausscheidung, selten Milzschwellung, Magen-Darm-Symptome, mehr oder weniger pathologische

Klinik / Eiweißbild und Serologie	Akute Virushepatitis	Chronisch interstitielle Hepatitis	Postnekrotische Lebercirrhose	Biliäre Cirrhose	Lupoide Hepatitis	Mechanischer Verschlußikterus
Gesamteiweiß	wenig vermindert ↓	vermindert ↓	vermindert ↓	stark erhöht ↑↑	erhöht ↑	normal
Albumine	vermindert ↓	vermindert ↓	vermindert ↓	vermindert ↓	vermindert ↓	wenig vermindert ↓
α_2-Globuline	normal	vermindert ↓	vermindert ↓	erhöht ↑	erhöht ↑	erhöht ↑
γ-Globuline	erhöht ↑	stark erhöht ↑↑	stark erhöht ↑↑	stark erhöht ↑↑	stark erhöht ↑↑	wenig erhöht ↑
β_2M-Globuline	normal	fakultativ erhöht ↑	fakultativ erhöht ↑	erhöht ↑	fakultativ erhöht ↑	∅
Auto-Ak.-Fixation in der Leber	Frühstadien: ∅ Spätstadien: (+)	++ (67%)	+ (47%)	+++ (75%)	+++ (~ 80%)	∅
Anti-nukleäre Ak.	∅	∅	∅	∅	++	∅
Rheumafaktoren	unter 1:64 schwach pos. (+)	über 1:64 +	bis 1:64 +	über 1:128 ++	über 1:64 +	∅
Kälte-Agglutinine	unter 1:32 schwach pos. (+)	über 1:64 +	bis 1:64 +	über 1:128 ++	über 1:64 +	fakultativ (+)
Auto-haem.-Ak.	∅	(+) nur bei haematolog. Komplikationen	++ bei haematologischen Komplikationen	++ bei haematologischen Komplikationen	++ bei haematologischen Komplikationen; LE-Zellen: ++	∅

Labilitätstests und Gerinnungsfaktoren, Bromsulphaleinretention, Transaminasen-Erhöhung nicht über 200-400 E.

Pathologisch-anatomische Kriterien: Fleckförmige Nekrosen, diffuse Leberzelldegeneration, daneben Regenerationserscheinungen, mesenchymale Knötchenbildung infolge histio-lymphocytärer Infiltrate mit Plasmazellvermehrung. Gallengangsproliferation, vorwiegend bindegewebige Umwandlung.

Postnekrotische Lebercirrhose:

Klinische Daten: Wie oben, Zunahme der Funktionsstörungen, kein Icterus, deutliche Verschiebung der Labilitätstests, Bromsulfaleinretention, Transaminasen-Erhöhung nicht über 200-400 E, geringe Erhöhung der alkalischen Phosphatase, Ascitesentwicklung.

Pathologisch-anatomische Kriterien: postnekrotische Lebercirrhose, Vermehrung der Gallengänge mit Cholostase, Regenerationszeichen, Reichlich Entzündungszellen in den portalen Feldern.

Biliäre Lebercirrhose:

Klinische Daten: Schwerer Icterus mit Pruritus, Schüttelfröste mit Fieber möglich, erhebliche Lebervergrösserung mit Milztumor, portale Hypertension, mögliche hämorrhagische Diathese, Hautpigmentierungen, Erhöhung der alkalischen Phosphatase und des Gesamtcholesterins, Labilitätsteste relativ wenig verändert, Prothrombin relativ lange normal.

Pathologisch-anatomische Kriterien: Gallenstase, Gallengangsproliferation, periportale Entzündungsvorgänge, perilobuläre Fibrose. Eindickung der Galle oder Steinbildung, interstitielle mononucleäre Zellinfiltrate, zunehmende Cirrhoseentwicklung.

Lupoide Hepatitis:

Klinische Daten: Hier sind etwas weitere Ausführungen notwendig, da das Krankheitsbild erst in den letzten Jahren in dieser Form abgegrenzt wurde und diese Abgrenzung nicht unumstritten geblieben ist. KUNKEL und später MACKAY und WOOD haben als lupoide Hepatitis ein Krankheitsbild abzugrenzen versucht, das ganz bevorzugt das weibliche Geschlecht in jüngeren Jahren befällt, wenngleich auch ältere Frauen erkranken. Die Erkrankung beginnt vielfach mit einer schleichenden, nicht zur Heilung neigenden Hepatitis und Gelbsucht, die in Schüben und Remissionen bis zur Ausbildung einer nodulären Cirrhose fortschreitet. Die ikterischen Schübe können von Gelenkerscheinungen begleitet sein. Das Bild eines generalisierten Lupus erythematodes ist hier also nicht gemeint, durchweg sind isolierte, chronisch entzündliche Lebererkrankungen beschrieben, die meistens allerdings mit Milztumor einhergehen. Histologisch finden sich interstitielle, vorwiegend plasmazelluläre Infiltrate in der Leber ohne Plasmazellvermehrung im Knochenmark. In der Behandlung sind Steroidhormone besonders wirksam, Übergänge zu ausgeprägten Cirrhosebildern sollen durch Steroidhor-

monverabfolgung unterbunden werden können. Eine Identität mit der Leberbeteiligung im Rahmen eines visceralen Erythematodes will man darum nicht annehmen, weil diese in der Regel ohne Icterus auftritt, kaum je Cirrhoseentwicklung zeigt und histologisch allenfalls focale Nekrosen, Verfettungen und lymphocytäre Infiltrate aufzuweisen pflegt. SIEGENTHALER ist neuerdings dafür eingetreten, die lupoide Hepatitis doch als die Frühmanifestation eines Erythematodes aufzufassen. Fieberschübe, Arthralgien, Empfindlichkeit für Sonnenlicht mit Hauterythem, weiter die Möglichkeit hämolytischer Krisen oder komplizierender Bronchopneumonien lassen in der Tat an eine Verwandtschaft zum Erythematodes denken, wie diese auch in den immunologischen Testen besonders deutlich wird. Das Bilirubin ist während der Schübe stark erhöht, Transaminasen und alkalische Phosphatase finden sich ebenfalls deutlich erhöht.

Pathologisch-anatomische Kriterien: Leberzellnekrosen, Plasmazelluläre und lymphozytäre Infiltrate, Entwicklung einer nodulären Cirrhose.

Fluoreszenzserologisch hat man die Gegenwart von Gamma-Globulinen in diesen Plasmazellen sichtbar machen können.

Aus der tabellarischen Gegenüberstellung lässt sich folgendes entnehmen:

Die Fixation von Auto-Antikörpern im Lebergewebe ordnet sich den chronisch-progredienten, im Leberinterstitium ablaufenden Entzündungsvorgängen ohne klinische Spezifität zu. Sie wird zum Symptom der chronisch entzündlichen Progredienz, ist differentialdiagnostisch aber nicht verwertbar.

Symptom der entzündlichen Aktivität ist auch das falsch-positive Auftreten der Rheumafaktoren, die sich im Titer bis 1:64 bei der Virushepatitis und ihren Folgezuständen finden, in höherem Titer nur bei der biliären Lebercirrhose und bei der lupoiden Hepatitis.

Nahezu ausschliesslich bei der lupoiden Hepatitis finden sich hoch positive antinucleäre Faktoren im Serum und das L. E. Zellphänomen. Diesen Befunden kommt also gewisse, wenn auch nicht ausschliessliche differentialdiagnostische Bedeutung in der Abgrenzung dieser Krankheitsformen zu. Verschlussicterusformen wurden nicht gesondert angeführt. Sie zeigen durchweg keine serologischen Phänomene. Positive serologische Befunde sprechen differentialdiagnostisch also gegen mechanische Verschlussicterusformen.

Der Grad der immunologischen Reaktionsbereitschaft ist proportional der Entwicklung der Dysproteinämie und auch dadurch Ausdruck der Schwere der Grunderkrankung bzw. ihrer Entwicklungstendenz.

b) *Immunhämatologische Komplikationen bei entzündlichen Lebererkrankungen:*

Mit dieser Gegenüberstellung sind die serologischen Untersuchungen bei entzündlichen Lebererkrankungen nicht erschöpft. Sie werden ergänzt durch solche immunologische Phänomene, die sich bestimmten, vor allem hämatologischen Komplikationen zuordnen: Die akute und

die chronische Hepatitis kann ebenso wie die postnekrotische Lebercirrhose gemeinsam mit einer immunhämolytischen Anämie auftreten. Klinisch findet sich dann eine persistente oder rezidivierende, aber nicht hochgradige Bilirubinerhöhung im Blut zusammen mit einer nicht einmal hochgradigen Anämie. Der Nachweis einer Mikrosphärocytose, einer Reticulocytenvermehrung, ggf. auch einer Resistenzverschiebung der Erythrocyten kennzeichnen das klinische Syndrom, dessen ätiologische Abgrenzung zur sog. *posthepatitischen Hyperbilirubinämie* durch den serologischen Befund ermöglicht wird. Letzterer ist sichter *nicht* hämolytischer Natur, sondern beruht auf einer fermentativen Störung im Bilirubinstoffwechsel.

Serologisch charakterisiert sind also nur echte, erworbene hämolytische Syndrome, die als solche durch entzündliche Lebererkrankungen ausgelöst werden. Man findet dabei

komplette Kälteagglutinine in oft hohem Titer und erhöhter Wärmeamplitude,

oder inkomplette Kälte-Auto-Antikörper,

oder komplette Wärme-Autohämolysine,

oder inkomplette Wärme Auto-Antikörper mit positivem Coombs-Test,

oder wie wohl meistens eine kombinierte Reaktion mehrerer dieser serologischen Phänomene gleichzeitig.

Dieser-Art immunologisch bedingte hämolytische Komplikationen sind bei der Virushepatitis selten, bei chronisch-interstitieller Hepatitis und Lebercirrhose häufiger, vor allem auch bei der lupoiden Hepatitis eine durchaus bekannte Komplikation. Verantwortlich für diese Form einer komplizierenden Auto-Antikörperbildung dürfte die Proliferation vorwiegend extrahepatischer, jedoch zur Antikörperbildung befähigter Zellclone sein. Die im Interstitium der Leber möglicherweise vorhandenen, ebenfalls zur Antikörperbildung befähigten lymphoiden und Plasmazellen können allenfalls für die Bildung von Gewebs-Auto-Antikörpern verantwortlich sein, die dann aber im Bereich der Leber selbst fixiert bleiben.

Andererseits liegt gerade bei der Lebercirrhose auch ein beschleunigter Abbau der Erythrocyten vor, so dass die Anämieentwicklung auch andere als serologische Ursachen haben kann. Immunologische Untersuchungen sind also notwendig, um die Eigenart echter, autoimmunhämolytischer Komplikationen zu begründen.

Inwieweit Funktionsstörungen auch der *Thrombocyten* und damit thrombopenische Zustände bei chronischer Hepatitis und Cirrhose ebenfalls immunologisch bedingt sein können, ist eine Frage, deren exakte Beantwortung wegen der technischen Schwierigkeiten bei der Bestimmung thrombocytärer Auto-Antikörper vielfach unbeantwortet bleiben wird. Zweifellos sind solche Vorgänge möglich und bei chronisch

interstitieller Entzündung vor allem nach Art der lupoiden Hepatitis belegt. Hämorrhagische Diathesen, wie sie gerade bei diesen chronisch entzündlichen Lebererkrankungen auftreten, brauchen demnach nicht allein durch eine Verminderung plasmatischer Gerinnungsfaktoren oder durch toxische Gefässwandschädigung bedingt zu sein (BREDDIN), sie können offenbar auch immunologische Mitursachen haben. Es steht ausser Zweifel, dass alle erwähnten Auto-Häm-Antikörper nicht nur klinisch diagnostisch von Interesse sind, sondern auch kausal für die Komplikationen verantwortlich.

c) *Die Leberbeteiligung im Rahmen extrahepatischer Primärerkrankungen mit hoher immunologischer Prägung:*

Auch diese Beobachtungen sind im Zusammenhang von grosser Wichtigkeit, weil sie uns aus der klinischen Empirie Hinweise dafür geben, dass Leberschädigungen durch immunologische Reaktionsabläufe im eigenen Organismus bedingt sein können.

GALBRAITH u. Mitarb. haben z. B. den Fall einer Myasthenia gravis, deren hohe immunologische Prägung durch die Fixation von Auto-Antikörpern in der Muskulatur zum Ausdruck kommt (STRAUSS), beobachtet, wo es als Spätkomplikation u. a. zur Entwicklung einer Lebercirrhose kam. Aus therapeutischer Indikation hatte man eine hyperplastische Thymusdrüse operativ entfernt. Etwa 1 Jahr nach dieser Operation kam es zur Ausprägung eines generalisierten Lupus erythematodes disseminatus und gleichzeitig zur Entwicklung einer ulcerösen Colitis. Zur selben Zeit wurden die ersten Hinweise auf eine Leberbeteiligung deutlich; nur diese schritt bis zum Vollbild der Lebercirrhose fort. Keine dieser Komplikationen war vor der Operation auch nur angedeutet. Aber sowohl der Lupus erythematodes wie auch die ulceröse Colitis sind ja durch autoimmunologische Vorgänge in hohem Masse geprägt. Mithin steht ein paralleler Mechanismus als Ursache der Leberbeteiligung zur Diskussion. Die Autoren diskutieren die Möglichkeit, dass durch die Thymektomie eine Schutzfunktion eliminiert wurde, in deren Folge autoimmunologische Prozesse in Freiheit gesetzt worden sind, die zu den erwähnten Komplikationen geführt haben.

Eine Leberbeteiligung bei *ulceröser Colitis* ist keineswegs selten (VINNIK und KERN, 1963). Dabei handelt es sich häufig um eine Pericholangitis, seltener sind chronisch-aktive Hepatitisformen. Wiederum etwas häufiger kommt es zur Entwicklung einer postnekrotischen Lebercirrhose, ganz ungewöhnlich ist eine LAENNEC-Cirrhose. Gerade die beiden letztgenannten Komplikationen einer ulcerösen Colitis treten bevorzugt bei jüngeren Frauen auf und entsprechen in einer Reihe von Zügen dem Bild der lupoiden Hepatitis. Einige der von VINNIK untersuchten Fälle zeigten ausser Colitis und lupoider Hepatitis eine Thrombocytopenie mit dadurch bedingter hämorrhagischer Diathese, Arthralgien, einen positiven Coombs-Test entsprechend der Fixation antierythrocytärer Auto-Antikörper und ein positives L. E.-Zellphänomen. Es ist gesichert, dass auch bei der Colitis ulcerosa Auto-Antikörper im entzündeten Darmbereich gebunden werden (vgl. bei BREGMANN und

bei HOFFMANN und VORLAENDER). Auch wissen wir, dass eine Colitis ulcerosa hier und da durch eine autoimmunhämolytische Anämie kompliziert sein kann (FUDENBERG und PERLEMANN). Daher liegt es nicht fern, auch für die obenerwähnte Leberbeteiligung bei ulceröser Colitis auto-immunologische Vorgänge verantwortlich machen zu wollen.

Im Zusammenhang sei nochmal auf die Möglichkeit der Miterkrankung der Leber bei Lupus erythematodes (TAFT und MACKAY) verwiesen, vor allem aber auch auf Fälle von Lebercirrhose, die im Verlauf des SJÖGREN-Syndroms zur Beobachtung gekommen sind. Das SJÖGREN-Syndrom steht auch nach immunologischen Gesichtspunkten dem Lupus erythematodes nahe, ja es gibt Autoren, die zwischen beiden Erkrankungen gar nicht mehr prinzipiell unterscheiden wollen. Die Leberbeteiligung bei primär-chronischer Polyarthritis oder Morbus Bechterew dürfte seltener sein, als in der Literatur erwähnt. Wir kennen aber auch Fälle, wo eine entzündliche Miterkrankung der Leber nach Art einer chronisch interstitiellen Hepatitis in der Folge einer Autoimmunthyreoiditis aufgetreten ist (MAC KONKEY). Bereits aus diesen wenigen kasuistischen Hinweisen möge verständlich sein, dass man vielfach geneigt ist, die sekundäre Leberbeteiligung im Rahmen seltener extrahepatischer Primärerkrankungen, die selbst durch abnorm hohe immunologische Reaktionsbereitschaft gekennzeichnet sind, ebenfalls auf analoge Vorgänge zurückzuführen.

Die klinische Bedeutung immunologischer Vorgänge bei Lebererkrankungen:

Eine kritische Bewertung hat hervorzuheben, dass die primäre Entstehung einer entzündlichen Lebererkrankrung durch auto-immunologische Vorgänge, die also im eigenen Organismus ablaufen, bis heute unbewiesen ist. Vor allem ist es nie gelungen, bei Tieren durch eine systematische Sensibilisierung gegen homologes Lebergewebe (unter Zusatz des FREUND'schen Adjuvants) oder gegen Kernnucleoproteine das Bild einer chronisch-interstitiellen Hepatitis oder das einer "lupoiden Hepatitis" zu erzeugen: Wohl führt diese Sensibilisierung zum Auftreten korrespondierender, homologer Antikörper, nicht aber zu einer Erkrankung, die auch nur annähernd mit den Verhältnissen beim Menschen vergleichbar wäre. Mit anderen Worten kennen wir bis heute kein experimentelles Modellbeispiel, aus dem die primär pathogene Auswirkung homologer, gegen bestimmte Zellen des Leberinterstitiums gerichteter Antikörper bewiesen wäre. Wenn eine Lebererkrankung also überhaupt durch ein immunologisches Geschehen ausgelöst werden soll, dann bleibt dies dem Einfluss exogener Fremdantigene mit nachfolgender heterologer Antikörpermildung vorbehalten. Das sind die eingangs beschriebenen, ebenfalls seltenen Fälle der *allergischen* Genese einer Leberschädigung.

Klinisch kommt ein auto-immunologischer Mechanismus, der also im eigenen Organismus entstehen und zur Wirkung kommen müsste, nur für die lupoide Hepatitis und für die zuletzt erwähnten Leberbe-

teiligungen im Rahmen extrahepatischer Primärerkrankungen mit abnorm hoher immunologischer Reaktionsbereitschaft (Autoimmunthyreoiditis; Lupus erythematodes disseminatus; ulceröse Colitis) in Frage. Es sind bisher aber doch nur klinisch empirische Argumente, die für einen solchen kausalen Zusammenhang sprechen. Exakte Beweise, wie sie in der Immunpathologie chronisch entzündlicher Nierenerkrankungen z. B. durch parabiotische Übertragungsversuche autoimmunologischer Prozesse auf gesunde Tiere unternommen worden sind, fehlen bei Lebererkrankungen, weil wir hier kein den menschlichen Verhältnissen auch nur annähernd vergleichbares Modell, z. B. einer plasmazellulären interstitiellen Hepatitis haben.

Noch ein anderer Gesichtspunkt ist entscheidend: Die ganz überwiegende Mehrzahl aller im vorhergehenden beschriebenen immunologischen Phänomene bei entzündlichen Lebererkrankungen tritt —parallel zur Dysproteinämieentwicklung— erst *sekundär* im Verlauf der Erkrankungen auf, die primäre Ätiologie all dieser Prozesse (Infektion oder Cholostase, vielleicht auch toxische Primärschädigung) hat mit immunologischen Vorgängen nichts zu tun. Unter den Verschiebungen der Serumeiweisskörper entspricht nur die Alpha-Globulinverminderung, wie sie für die Entwicklung einer postnekrotischen Lebercirrhose typisch ist, einer primären Proteinumsatzstörung in der Leber selbst (MÄRKI). Die Vermehrung der Gamma-Globuline und die der $Beta_2$-Makro-Globuline erfolgt überwiegend extrahepatisch in lymphoiden Reticulumzellen und Plasmazellen, vor allem aber *reaktiv*. Damit werden auch die zirkulierenden Antikörper ganz überwiegend extrahepatisch und reaktiv ausgebildet und können schon dadurch nicht Primärursache der zur Diskussion stehenden Lebererkrankungen sein. Allenfalls inkomplette Antikörper, die nie in die Zirkulation kommen, sondern an lymphoiden Zellen fixiert bleiben, mit diesen aber an den Ort des entzündlichen Geschehens transportiert werden können, mögen zum Teil in den plasmazellreichen Infiltraten der Leber selbst gebildet werden. Aber auch diese Plasmazellvermehrung muss durch einen anderen Primärprozess (meist Infektionen) ausgelöst sein.

Andererseits ist es gerade für die chronisch progrediente Krankheitsentwicklung charakteristisch, dass die Gamma-Globuline und ggf. auch die $Beta_2$-Makro-Globuline oft beträchtlich vermehrt bleiben. Nach MÄRKI ist das die Folge einer verminderten Abbaukapazität der Leber. Es ist also nur logisch, wie der im Vorhergehenden wiedergegebenen Kasuistik auch leicht zu entnehmen, dass mit Erhaltenbleiben des Gamma-Dysproteinämietyps auch alle Antikörperbildungsvorgänge langfristig gegenwärtig und reaktionsbereit bleiben müssen.

Ähnlich den Verhältnissen bei den zum Rezidiv neigenden Formen der Myocarditis rheumatica oder der chronischen Glomerulonephritis wird die Ausbildung aller jener immunologischer Vorgänge, die ohne Fremdantigenwirkung entstehen, entweder durch primäre Infekte oder durch die Entzündung im Lebergewebe erst vermittelt. Aus experimentellen Untersuchungen von ESPINOZA und INSUNZA wissen wir, dass analoge Antigenwirkungen auch in der Folge toxischer Leberschädigun-

gen, etwa nach Tetrachlorkohlenstoff oder Chloroform auftreten und serologisch exakt erfasst werden können.

Für den Kliniker ergibt sich also die Frage, inwieweit das klinische Bild einer bestimmten, entzündlichen oder toxischen Lebererkrankung und seine Verlaufseigenart durch diese immunologischen Sekundärvorgänge geprägt werden kann.

Vor allem zwei klinische Beobachtungen sprechen für kausale Wechselbeziehungen zwischen entzündlicher Progredienz und immunologischem Geschehen: Aus der Kasuistik wurde deutlich, dass alle immunologischen Phänomene parallel zu einer progredienten Entwicklung der entzündlichen Leberveränderungen immer deutlicher hervortreten. Umgekehrt können sowohl Steroidhormone wie 6-Mercaptopurine (lupoide Hepatitis!) das immunologische Geschehen eindeutig bremsen und dabei gleichzeitig eine klinische Besserung oder zumindest die Begrenzung des Etzündungsgeschehens einleiten. Auch nach fluoreszenzoptischen Untersuchungen findet sich die Antikörperfixation in der entzündlich veränderten Leber durchweg der klinischen Progredienz des Leidens zugeordnet, selbst wenn sie schon zu Beginn der entzündlichen Veränderungen nachgewiesen worden sein sollte. Für alle immunhämatologischen Komplikationen entzündlicher Lebererkrankungen ist eine Fixation von Auto-Antikörpern an Blutzellen als die Ursache der komplizierenden klinischen Symptome bewiesen.

Ohne Zweifel aber gelten für die sehr verschiedenen Antikörperbildungsvorgänge bei entzündlichen Lebererkrankungen auch sehr verschiedene Massstäbe bezüglich der Möglichkeiten einer kausalen Einflussnahme: Rheumafaktoren, aber auch antinucleäre Antikörper sind mit weitgehender Sicherheit von rein diagnostischem Interesse, als solche der entzündlichen Progredienz oder einer bestimmten Krankheitsentwicklung zwar zugeordnet, doch sicher nicht kausal für diese verantwortlich. Beide Faktoren wurden versehentlich bei Bluttransfusionen oder von erkrankten Müttern auf ihre Kinder übertragen, ohne Organveränderungen zu provozieren.

Anders liegen die Verhältnisse für cytoplasmatische Antikörper, die an Mitochondrienfraktionen gebunden werden: Auch sie können bei intakter Zellmembran keine Zellschädigung bewirken, weil ihnen dann nämlich der Eintritt in die Zelle verwehrt bleibt. Wenn aber die Permeabilitätsverhältnisse im entzündlichen Geschehen aufgelockert und verändert sind, dann können diese Antikörper auf die Mitochondrienfraktion und alle hier lokalisierten Fermentvorgänge Einfluss nehmen. Modellversuche von FISCHER haben gezeigt, dass infolge einer solchen Einflussnahme, die die Mitwirkung einer bestimten Complementkomponente voraussetzt, Zelluntergang eintreten kann.

POPPER hat gezeigt, dass antiductuläre Auto-Antikörper, die also ausschliesslich im basophilen Cytoplasma gewucherter Gallenkapillarzellen gebunden werden und damit organspezifisch sind, auch in jenen Phasen der Erkrankung nachweisbar bleiben, wo histologische Aktivitätszeichen nicht mehr oder noch nicht ausgeprägt sind. Die beiden letzterwähnten Befunde legen es nahe, an eine immunologische Mitursache der chronisch entzündlichen Progredienz bei Lebererkrankungen

zu glauben. Sie könnten eine klinische Entwicklung erklären, für die wir andere Ursachen bisher nicht kennen. Letzte gültige Beweise fehlen auch hier, weil wir auch hier bisher kein vergleichbares Modell mit parallelen Auswirkungen autoimmunologischer Vorgänge kennen. Dennoch besteht die Möglichkeit, dass sich vor allem die auto-immunologischen Vorgänge nicht nur als diagnostisch interessantes Charakteristikum der chronisch entzündlichen Krankheitsentwicklung zuordnen, sondern dass sie auch kausal dafür verantwortlich oder mitverantwortlich sind.

ZUSAMMENFASSUNG.

Die in der Einleitung formulierten Fragen lassen sich demnach wie folgt beantworten:

I. Entzündliche Lebererkrankungen können durch exogene Allergenwirkungen ausgelöst werden, hier vor allem im Rahmen der Serumanaphylaxie oder der medikamentösen Allergie. Klinisch tritt diese Leberschädigung als hepatozellulärer Icterus oder aber in Form einer allergischen Cholangiolitis in Erscheinung. Sicher allergisch bedingte Leberkrankheiten sind in beiden Formen jedoch seltener, als vielfach angenommen.

II. Im Verlauf jener entzündlichen Lebererkrankungen, die sicher *keine* allergische Primärgenese haben, treten ebenfalls zahlreiche immunologische Phänomene verschiedener klinischer Wertigkeit auf: Wichtigster Befund dabei ist die Fixation homologer Auto-Antikörper im Lebergewebe, der sich vordringlich den chronisch progredienten Entzündungsformen zuordnet.

Ferner können Rheumafaktoren falsch positiv im Serum messbar werden. Wir kennen sogar falsch positiv erhöhte Anti-O-Streptolysintiter infolge Lipoidvermehrung im Serum mit Hämolysehemmung, ja wir kennen sogar "falsch positive" Wassermann-Reaktionen.

Bevorzugt bei der lupoiden Hepatitis können weiterhin antinucleäre Serumfaktoren und selbst das L. E. Zellphänomen positiv werden.

Diese so verschiedenartigen Antikörperbildungsvorgänge ordnen sich entweder den 7-S-Gamma-Globulinen (Mehrzahl der Auto-Antikörper gegen Leber; antinucleäre Serumfaktoren; Auto-Häm-Antikörper bei Krankheitskomplikationen),

oder den 19-S-Globulinen (Rheumafaktoren; Kälte-Antikörper; ein kleiner Teil cytoplasmatischer Auto-Antikörper)

zu und kommen wie diese *reaktiv zum Krankheitsgeschehen* zur Ausprägung. Auto-Häm-Antikörper ordnen sich durchweg den entsprechenden hämatologischen Komplikationen zu.

III. Die überwiegende Mehrzahl der 19-S-Faktoren hat eine wichtige diagnostische Bedeutung als Symptom der entzündlichen Aktivität bzw. Progressionstendenz. Antinucleäre Faktoren vom Typ 7 S können in Zuordnung zur klinischen Symptomatologie für die Sonderform der lupoiden Hepatitis sprechen. Alle mechanischen Verschlussicterusfor-

men bleiben serologisch durchweg negativ, so dass die positiven serologischen Befunde, insbesondere dann, wenn mehrere dieser Antikörper gleichzeitig auftreten, gegen mechanischen Verschluss sprechen müssen.

Alle Auto-Häm-Antikörper, vor allem die antierythrocytären Antikörper, sind kausal für die hämatologischen Komplikationen verantwortlich und diagnostisch in der Abgrenzung zu der gutartigen "posthepatitischen Hyperbilirubinämie" verwertbar.

Ob die chronisch entzündliche Progredienz einer Lebererkrankung oder die sekundäre Leberbeteiligung im Rahmen extrahepatischer Primärerkrankungen mit hoher immunologischer Prägung (Auto-Immunthyreoiditis, Lupus erythematodes disseminatus, Colitis ulcerosa) durch die Fixation von Auto-Antikörpern in der Leber selbst kausal bedingt oder mitbedingt wird, ist eine letzlich noch offene wissenschaftliche Frage. Die zeitliche wie quantitative Zuordnung autoimmunologischer Phänomene zur progredienten Krankheitsentwicklung wie die gleichsinnige Beeinflussbarkeit beider durch Steroidhormone oder 6-Mercaptopurine können für pathogenetische Zusammenhänge sprechen.

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PATHOGENETIC MECHANISMS IN ALLERGIC RENAL AND VASCULAR DISEASES

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The implication of allergic or immunologic mechanisms in a number of renal and vascular diseases such as glomerulonephritis and urticaria began with clinical observations made early in this century. It was the similarity between serum sickness, an assumed immunologic disorder, and these clinical diseases that first suggested an allergic etiology of the latter. In the 1930's and 40's, pathologists added the entities lupus erythematosus, rheumatoid arthritis, rheumatic fever and polyarteritis, to the list of serum sickness-like diseases. Within the past six to seven years, experimental observations have gone a long way in defining the pathogenetic mechanisms operative in serum sickness and have suggested that similar mechanisms may be operative in the clinical entities which resemble it [1]. These observations, from a number of different experiments, have all pointed to the pathogenic role of antigen-antibody complexes immunologically unrelated to the tissues which are injured. In this sense, the proposed pathogenesis of complex induced disease is quite different from that postulated in the tissue specific, autoimmune diseases of thyroid, brain, etc. where specific anti tissue antibodies or sensitized cells are presumed to mediate the injury.

Antigen-antibody complexes, themselves made up of innocuous molecules, apparently can act as potent anaphylactic and phlogogenic stimuli under appropriate conditions. Germuth observed that antigen-antibody complexes were capable of inducing systemic anaphylaxis in guinea pigs and that complexes formed in moderate antigen excess were the most effective [2]. It was found by Ishizaka and associates that complexes capable of increasing vascular permeability were formed from antibody with affinity for tissues and themselves had such an affinity [3] and that they were able to interact with complement [4]. Further, it was seen that the activity of complexes depended on the properties of the antibody, not the antigen, and that the activity of soluble comple-

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xes was associated with the change in the properties of optical rotation of the complex [5]. These findings led to the proposal that the configuration of antibody molecules brought into close apposition in an immunological complex was altered, probably in the fraction III segment of the molecules and this altered configuration was responsible for the complexes changed optical rotation and their ability to fix complement and induce a phlogogenic stimulus. They were able to show by heat aggregating or chemically linking human gamma globulin that the resulting aggregates had affinity for tissues, could fix complement and showed the biological activities just as did antigen-antibody complexes [6]. These observations stress the importance of the antibody or gamma globulin part of the complex and suggested that gamma globulin complexes induced on non-immunological bases might also be pathogenic.

Gamma globulin containing complexes appear to produce their biological effects via a number of interactions with both humoral and cellular elements of the host. At a molecular level the complexes interact with fresh serum constituents to produce active substances such as esterase from C'1 [7], fibrinolysin [8], anaphylatoxin [9], and peptides such as bradykinin. These molecular reactions could, and probably do, occur both systemically in the circulation and locally in tissue lesions. At a cellular level complexes either directly or indirectly have the ability to: 1) cause mast cell degranulation and presumably liberate pharmacologically active substances [10], 2) react with and agglutinate leukocytes and platelets [11], 3) increase the coagulability of whole blood [12], 4) cause contraction of smooth muscle [13], 5) increase vascular permeability [14], 6) induce vascular endothelial proliferation [15 a b], 7) attract polymorphonuclear lekocytes by chemotaxis [16], and 8) preciptate in certain anatomical sites causing a hyalin type of degeneration [17].

The extreme pathogenicity of antigen-antibody complexes and their modes of action can be best appreciated from the study of several experimental diseases in which they appear to play an essential role. Three examples will illustrate the spectrum of tissue lesions which may be induced by antigen-antibody complexes, depending upon the logistics of the immunologic reaction involved. First, the simplest complex induced lesion in the Arthus reaction causing localized, acute necrotizing vasculitis. Essential to the full development of this lesion is the formation of relatively large amounts of antigen and antibody complexes in blood vessel walls [18]. In order to so locate the reaction, one of the reactants, either antigen or antibody, must be in the circulation and the other injected locally. Early in the reaction antigen and antibody diffuse toward each other, meeting and precipitating in the vessel walls. This antigen-antibody complex is apparently chemotactic for polymorphonuclear leukocytes and within a few hours these cells infiltrate the involved vessels causing damage or destruction. The polymorphonuclear leukocytes phagocytize the antigen-antibody complexes and appear to catabolize them and/or carry them away from the site of reaction. In the absence of polymorphonuclear leukocytes in the circulation, immunologic events essential to an Arthus reaction may transpire but the inflammatory necrotizing reaction fails to develop. If polymorphonuclear

leukocytes are removed from the circulation of an immunized rabbit by nitrogen mustard and antigen is injected intradermally, the antigen-antibody precipitate forms sub-endothelially in the local vessels without significant tissue damage. These antigen-antibody precipitates persist in the vessel wall in the absence of polymorphonuclear leukocytes and with the return of the latter to the circulation, a devastating Arthus reaction develops [18]. Thus, in this disease complexes appear to exert their pathogenic effect by attracting large numbers of polymorphonuclear leukocytes which, in the course of removing and/or degrading the complexes, cause inflammation and necrosis.

More complex, but probably much closer to the situation in systemic human disease than the Arthus reaction, is classical "one-shot" serum sickness. In this disease either in experimental animals or in humans, a single large dose of foreign serum or purified serum protein is given to the subject. The antigen persists in the circulation until antibody formation occurs at which time antigen-antibody complexes are formed in the circulation [15a]. This occurs first in an extreme antigen excess environment, predisposing to the formation of small complexes capable of remaining in the circulation, but incapable of fixing C' or injuring tissue. As the amount of antibody formed increases, the antigen-antibody complexes become larger and are finally removed from the circulation. Coincident with the formation of larger antigen-antibody complexes in the circulation there is a fall in the serum complement level and the appearance of acute inflammatory lesions in the kidneys, arteries, heart, and joints strongly reminiscent of the lesions of acute glomerulonephritis, lupus erythematosus, polyarteritis nodosa, rheumatic fever, and rheumatoid arthritis. These lesions vary from organ to organ but prominent in most are endothelial proliferation, increased vascular permeability, a variable polymorphonuclear leukocyte infiltration, and tissue damage.

Consistent with the proposed pathogenetic role of antigen-antibody complexes in these lesions it has been possible to localize concentrations of antigen, host C' and host gamma globulin in the developing lesions [1, 15a]. Not only do these substances deposit and become concentrated in the lesions of serum sickness, but the passive administration of antigen-antibody complexes to normal animals may produce arteritis, endocarditis and glomerulonephritis [19]. It cannot be determined whether the initial local phlogogenic stimulus in serum sickness lesions results from systemic liberation of active humoral agents which act locally or from the fortuitous focal deposition of small amounts of complexes. However, it is likely that the accumulation of complexes from the circulation into the developing lesion, as a result of increased vascular permeability, etc., causes a snowballing inflammatory reaction. With the combination of all antigen into complexes and its subsequent elimination from the circulation, the inflammatory lesions in all sites rapidly resolve and only occasional microscopic scars remain.

If antigen-antibody complexes are involved in the chronic clinical entities mentioned above, it seems likely that experimental conditions designed to keep small amounts of complexes present in the circulation

for long periods of time might produce chronic, progressive disease. By injecting intravenously small amounts of heterologous serum proteins daily into rabbits for many months, it has been possible to produce a situation in which complexes are present in the circulation most of the time [17]. Rabbits injected with small amounts of foreign serum protein daily make one of three kinds of response: 1) they produce large amounts of antibody which is capable of combining with all the injected antigen and promptly eliminating it from the circulation; 2) they fail to make any antibody, in which case the antigen which is itself non-toxic persists in the circulation for long periods of time, and 3) they make small amounts of antibody, sufficient to form antigen-antibody complexes in a situation of antigen excess, but insufficient to remove the antigen from the circulation. In these animals, soluble antigen-antibody complexes can be demonstrated in the circulation most of the time. Those animals making amounts of antibody large enough to eliminate the antigen promptly and those animals making no antibody showed no evidence of a chronic disease resulting from the antigen administration. On the other hand, those rabbits making a small antibody response which resulted in the persistence of complexes in the circulation developed a chronic membranous glomerulonephritis quite different from that seen in acute "one-shot" serum sickness [17]. The development of this disease depended entirely upon the relative amounts of antigen and antibody and the disease could be turned off or on by changing the dose of antigen to eliminate circulating complexes. If the dose of antigen was either lowered to allow continual antibody excess or raised to give an infinitely large antigen excess, progress of the disease stopped. If the dose was again returned to a level resulting in moderate antigen excess, the disease progressed. This chronic membranous glomerulonephritis was similar morphologically and fuctionally to chronic membranous glomerulonephritis or lupus nephritis seen in the human. The glomerular capillary basement membranes were greatly thickened. In the instance where no endothelial proliferative response was observed, the disease appeared much more degenerative than inflammatory by usual morphologic criteria. Host gamma globulin, host complement and the injected antigen were readily demonstrable in the thickened basement membrane. By electron microscopy, a lumpy dense deposit was seen along the outer aspect of the basement membranes corresponding to the antigen, gamma globulin and complement rich deposits visualized by the fluorescent antibody technique. Once in this site the immunologic reactants and the morphologically demonstrable deposits persisted for long periods—as much as one year after cessation of antigen injection—with persistence of associated renal malfunction.

Since the circulating complexes in either "one-shot" or daily injection serum sickness bear no known immunologic relationship to the kidneys or blood vessels or other structures injured, the factors predisposing these tissues to injury by the complexes are apparently non-immunologic. In the prolonged daily exposure to low levels of circulating complexes, the kidneys were the only organs injured while in "one-shot" serum sickness, with larger amounts of complexes present for a brief

period, heart, vessels and joints also suffered lesions. In all instances the tissues injured serve as filters of serum components. The great vulnerability of the kidneys in the presence of low levels of complexes may be related to the extensive renal blood flow and the normal filtering function of this organ. The exclusive concentration of complexes along the outer aspect of the basement membrane of the renal glomeruli is also probably determined by anatomic and physiologic peculiarities of the kidney. Some complexes apparently traverse the basement membrane and the epithelial cells. It may be that as the complexes progress through the basement membrane, they tend to aggregate and become less soluble as a result of a reduction of excess antigen in the environment. Once deposited outside the basement membrane, the complexes appear to be sequestered from cells capable of degrading them.

Study of these experimental diseases suggest that the nature of the injury induced by antigen-antibody complexes depends in large part on the quantitative relationship of the two reactants and is quite independent of the immunologic characteristics of the antigen and the tissues injured. First, contrary to the customary situation in acute anaphylactic reactions and "one-shot" serum sickness, where good antibody producers are the most vulnerable, chronic disease related to prolonged exposure to small amounts of endogenous or exogenous antigens may be more likely in poor antibody formers where soluble antigen-antibody complexes would be formed in antigen excess. Second, it appears that complexes are capable of inducing either acute inflammatory and necrotizing lesions or chronic degenerative lesions, depending upon the logistics of the reaction. Third, sites of localization of complexes and therefore the injury they cause appear to be determined by the physical character and perhaps quantity of complexes and upon local anatomic and phyisiologic factors in the tissue and not upon any immunologic relationship between complex and tissue.

How can these experimental observations best be applied to the study of human disease? To date, the only means of observing complexes directly in human disease is the insensitive method of analytical ultracentrifugation of the sera. Using this technique a variety of gamma globulin containing complexes, behaving much as antigen-antibody complexes, have been found in the sera of patients with a number of diseases, the classical example of which is rheumatoid arthritis [20]. Since rheumatoid factors, both 7 and 19S, behave like antibody to the patient's own gamma globulin, it is not surprising that such complexes are present in this disease. No invariable clinical features have been associated with the presence of complexes in rheumatoid patients, but such individuals are prone to severe joint disease and to vascular disorders. However, membranous renal disease is not a common feature of these patients. Less extensive study of other diseases has suggested the presence of gamma globulin containing complexes in the sera of some patients with systemic lupus, purpura, untreated subacute bacterial endocarditis, macroglobulinemia, Sjogren's disease, idiopathic pulmonary fibrosis and several less well defined entities [1]. Intermediate size complexes (9-15S) were frequently present in large amounts in rheumatoid patients with pul-

monary fibrosis and in each of four patients with Sjogren's disease and pulmonary fibrosis.

The concept of antigen-antibody complex induced disease is appealing since it is based upon the well demonstrated tendency for gamma globulin molecules to form biologically active complexes, either as a result of immunologic interaction or as a result of physical-chemical alteration of the gamma globulin molecules themselves. Thus, potential etiologic agents capable of inducing complex formation may include not only the antigen-antibody interaction but abnormal physiological denaturation of endogenous proteins and reactions of endogenous proteins with exogenous physical-chemical factors, including infectious agents. Those structures involved in filtering circulating fluids, i. e., blood vessels, especially glomerular capillaries, endocardium and synovium, would be excepted to be the most likely sites of accumulation of circulating complexes and therefore the most likely sites of complex induced injury. However, in order to understand the pathogenesis of these lesions more completely, it will be necessary to define the peculiar anatomic and physiologic factors which seem to be important in localizing the injury induced by this relatively non-specific pathogen. If complexes are shown to be important in human disease, we may have a unique therapeutic opportunity, for in some situations it may be possible to manipulate and eliminate the circulating complex and therefore, disease, according to time honored immunochemical or physical-chemical procedures performing, if you will, immuno-chemical titrations *in vivo* with the condition of the patient serving as the indicator.

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ALLERGY IN NEUROLOGICAL DISEASE

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Allergy in disease may occur as an incidental phenomenon arising from the effects of such processes, and in these circumstances may result from tissue breakdown, or else it may play an active part in the production of lesions or disturbance of function forming part of the disease process. During the last decade the concept of autoimmunity as forming a most important part of the study of allergic phenomena, has been of increasing importance particularly in relation to many chronic inflammatory diseases whose aetiology has proved most baffling. These concepts arise both from clinical observations and also from experimental work.

The discovery of a simple means of causing experimental allergic encephalitis based on the FREUND adjuvant technique by KABAT, WOLF and BEZER (1947) and MORGAN (1947) has given a tremendous impetus to the experimental study of encephalitis and also led to the discovery of experimental allergic neuritis (WAKSMAN & ADAMS, 1956) and adrenalitis (COLOVER and GLYNN).

Further work on the fractions in acid-fast bacilli by COLOVER and CONSDEN (1956) and COLOVER (1958) showed that the capacity to produce encephalitis was possessed by very resistant cell wall material obtained from heat-killed tubercle bacilli and also in a Wax D fraction prepared from unheated tubercle bacilli by E. LEDERER and co-workers. The latter fraction had undergone a considerable amount of purification and was still active. Both the wax material and cell wall residues contained a peptide (composed of glutamic acid, diaminopimelic acid and alanine), mycolic acid and also some polysaccharide. The synthesis of such active agents has not yet been achieved. COLOVER (1958) also showed that it was possible to combine these "purified" bacillary fractions with "purified" bovine spinal cord extracts and used these materials to induce the encephalitis.

Further work on the central nervous system (C. N. S.) components responsible for these reactions has shown that various active fractions may be obtained from nervous tissue. In a paper read to this society

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(Proceedings of Fourth European Congress of Allergy) COLOVER showed that encephalitogenic extracts which had been prepared by Marion Kies of Bethesda of the acid extract "type" did not produce visible skin reactions in animals in whom encephalitis had been induced. Circulating antibodies to this type of material prepared from human tissue have been found by Field, Caspary and Ball not only in patients suffering from multiple sclerosis but also in general paralysis of the insane and even normal individuals. Subjects who had been vaccinated with B. C. G. also showed an increased response in this direction.

THOMPSON, KIES and ALVORD (1963) have found that a fraction residing in myelin is active in producing encephalitis and this material is a non-dialyzable lipoprotein complex. It is completely extractable from myelin.

Since a raised gamma globulin in the cerebrospinal fluid (C. S. F.) may be indicative of an allergic reaction in the C. N. S., COLOVER, FEIN-

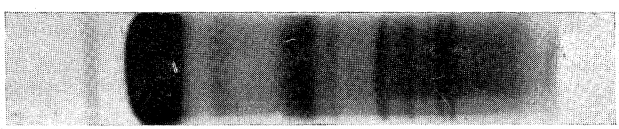

Figure 1.—Electrophoresis on polyacrylamide gel showing separation of the spinal fluid proteins into numerous discrete bands. The prealbumin and albumin components are seen an the left-hand side of the figure. On the right-hand side are many bands and it is in this region that one might expect the appearance of some of the gamma globulin material and also haptoglobins. These particular components, however, have not yet been identified in any detail. This fluid came from a patient who clinically showed recurrent attacks of arachnoiditis. The gel has been stained with naphthalene black.

BERG, TEMPLE and TOOLEY recently studied its measurement and found that there may be quite large discrepancies in the results obtained depending on whether the measurement was done by an immunological or electrophoretic technique. The meaning of these discrepancies has not yet been solved. Recent work on C. S. F. proteins by electrophoresis on polyarylamide gel shows that in some cerebrospinal fluids well over 20 separate bands may be identified (see illustration Figure I, showing the C. S. F. from a patient with arachnoiditis).

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THE INCIDENCE OF AUTO-ANTIBODIES IN THE NORMAL POPULATION (*)

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Our better understanding of auto-immune disorders is partly due to the introduction and the improvement of methods for detecting their presence, such as the tanned red cell agglutination test, the precipitin reaction, the complement-fixation test, the fluorescent antibody technique etc., and partly to recent biological research such as that on encephalomyelitis, experimental thyroiditis and runt disease which have advanced our knowledge of immunologically produced tissue lesions. This has led to the discovery of auto-antibodies on an ever widening scale and has considerably enhanced our hypotheses on their pathogenic importance.

Within the wide range of phenomena covered by the term pathogenic immunoreactions, auto-immunoreactions form a group by themselves. We can distinguish *immediate* reactions with seric antibodies as for example the oedematous reactions and *delayed* reactions in which the antibody is contained within the limphoid cells. In these we have the tuberculin-type reaction and the eczematous reaction. Finally there are *intermediate* or *combined* forms in which antibodies are present both in the serum and in the cells. These include the immunological reactions to grafts and the auto-immunological reactions.

Recent research tends to show that the presence of circulating auto-antibodies is not in itself sufficient to produce an auto-immune disease which is both progressive and self-sustaining. For such a disease to become manifest it is necessary for the patient to develop an alteration of the mechanisms of delayed sensitivity. The diagram shows how we interpret the three factors that must combine to produce an auto-immune thyroiditis (figure 1). They are the humoral factors represented by the circulating auto-antibodies; the cellular factors responsible for the delayed sensitivity and finally a particular lability of the so called shock organ as a local predisposition to the reaction. There is usually an interplay of these factors among themselves but individually, even if they persist

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(**) Institute of "Patologia Speciale Medica e Metodologia Clinica" of University of Sassari (Italy), directed by Prof. U. Serafini.

for a very long time, they cannot produce an auto-immune thyroiditis. For this to happen they must all be present simultaneously.

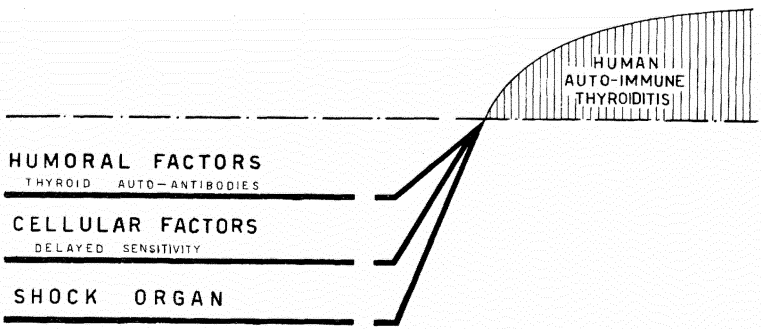

Figure 1.

It follows that we must distinguish clearly between *auto-immunization* and the mere *presence of circulating auto-antibodies;* the latter having in itself little or no pathological significance.

The chart shows how *in disease* auto-antibodies may be clinically

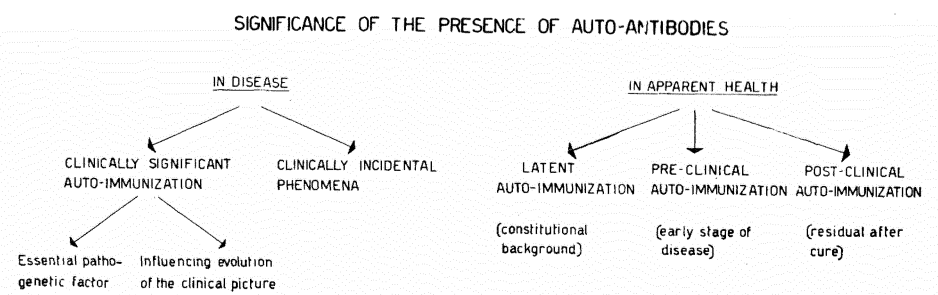

Figure 2.

significant —whenever the criteria proposed by Witebsky be fulfilled— as an essential pathogenic factor, influencing the evolution of the clinical picture, or merely incidental. *In apparent health* we may find a *latent auto-immunization* as an expression of a constitutional background as has been demonstrated by genetic studies on the families of patients suffering from Hashimoto's disease and rheumatoid arthritis. In other cases we may find *pre-clinical auto-immunization* in the very earliest stages of the disease and finally a *post-clinical auto-immunization* after the disease has subsided (figure 2). Thus we have to exercise the greatest caution in applying the presence of circulating auto-antibodies to clinical considerations.

* * *

Now while it is true that extensive studies have been undertaken to find circulating auto-antibodies, this has usually been done with reference

to a definite clinical entity and not nearly to the same extent in a population in apparently excellent health; moreover such studies have usually been limited to the search for a single auto-antibody.

The incidence of positive findings has been variously assessed by such authors as HACKETT ET AL. who found an incidence of 18 %, HILL of 10 %, GOUDIE ET AL. of 6.8-16 % for thyroid auto-antibodies; TAYLOR ET AL. of 5-16 %, MARKSON and MOORE of 4.5 % for gastric antibodies; BLOCH and BUNIM of 3 %, BARTFELD of 0.6-1 %, PLOTZ and SINGER of 0.8 %, MALTARELLO and CARCASSI of 3.81 % for the rheumatoid factor; ALEXANDER ET AL. of 4 %, SELIGMANN ET AL. of 2 % for the antinuclear factor; GAJDUSEK of 3 %, ASHERSON and BROBERGER of 0.5 % for the auto-immune complement fixation.

We have in recent years studied this problem in 1,430 healthy persons in whom we simultaneously looked for the following auto-antibodies: antithyroglobulin, second colloid antigen (CA2) antibodies, thyroid microsomal fraction antibodies, gastric antibodies which are all organ-specific; antinuclear factor(s) (A. N. F.), rheumatoid factor(s) (R. F.), auto-immune complement-fixation (A. I. C. F.) antibodies which are non-organ-specific.

Owing to the large number of persons tested we have been able to correlate the incidence to sex and age. In our series there were 804 males and 626 females. Ages ranged from 1 to 89 years.

Most of our subjects were blood donors, students and military personnel from the Provinces of Sassari (Sardinia) and Rome. The sera of the higher age groups were collected in homes from persons who had no illness of which the medical staff of the institutions was aware.

We used the following *methods* to demonstrate the auto-antibodies: tanned red cell agglutination test according to FULTHORPE and Coll. (1961) for the antithyroglobulin antibodies; the fluorescent antibody test according to the method of COONS and KAPLAN (1950) using unfixed thyroid sections for the thyroid microsomal fraction antibodies; the fluorescent antibody test, using alcohol-fixed thyroid sections according to BALFOUR ET AL. (1961) for the CA2 antibodies: the fluorescent antibody test and the complement-fixation test according to TAYLOR ET AL. (1962) for the gastric antibodies; Ling's method for the R. F.; the fluorescent antibody test according to the method of WEIR and HOLBOROW (1961) for the A. N. F.; complement-fixation test using saline homogenates of fresh rat liver and 2 M. H. D. of complement for the A. I. C. F.

Table I shows the *incidence of auto-antibodies* found to be present in the series of 1,430 subjects in percentages. Antithyroglobulin in 6.85 %; anti-CA2 5.66 %; thyroid microsomal fraction antibodies 3.70 %; gastric antibodies 5.03 %; rheumatoid factor 4.82 %; A. N. F. 0.90 %; A. I. C. F. 1.60 %. The total number of tests performed (7 in each case) was 10,010 and among these there were 409 positive reactions equal to 4.08 %. Of all persons examined, 347 (24.26 %) had a positive reaction for one or more auto-antibodies and of these 298 (85.59 %) were positive only for a single auto-antibody.

The degree of positivity established by tanned red cell agglutination test and the complement-fixation test was generally low. Though still

Tab. I.—Incidence of positive serological reactions for organ-specific and non-organ-

Age distribution (by decades)	Number of subjects	Positive serological							
		THYROID AUTO-ANTIBODIES						Gastric Antibodies	
		Thyroglobulin Antibodies		CA2 Antibodies		Microsomal Antibodies			
		No.	%	No.	%	No.	%	No.	%
	Males								
1-10	34	0	0	0	0	0	0	0	0
11-20	100	2	2.00	1	1.00	0	0	1	1.00
21-30	155	2	1.29	3	1.93	3	1.93	4	2.58
31-40	150	3	2.00	5	3.33	4	2.66	3	2.00
41-50	109	2	1.83	5	4.58	5	4.58	6	5.50
51-60	117	6	5.12	7	5.98	5	4.27	6	5.12
61-70	56	5	8.92	6	10.71	3	5.35	5	8.92
71 +	83	16	19.27	11	13.25	8	9.63	8	9.63
Total males	*804*	*36*	*4.47*	*38*	*4.72*	*28*	*3.48*	*33*	*4.10*
	Females								
1-10	32	1	3.12	0	0	0	0	0	0
11-20	100	3	3.00	1	1.00	1	1.00	2	2.00
21-30	104	7	6.73	4	3.84	1	0.96	5	4.80
31-40	95	8	8.42	5	5.26	2	2.10	4	4.21
41-50	87	9	10.34	6	6.89	3	3.44	6	6.89
51-60	95	13	13.68	14	14.73	6	6.31	10	10.52
61-70	53	11	20.75	6	11.32	7	13.20	8	15.09
71 +	60	10	16.66	7	11.66	5	8.33	4	6.66
Total females	*626*	*62*	*9.90*	*43*	*6.86*	*25*	*3.99*	*39*	*6.23*
TOTAL	**1430**	*98*	**6.85**	*81*	**5.66**	*53*	**3.70**	*72*	**5.03**

specific auto-antibodies 1.430 healthy subjects grouped according to sexes and ages.

reactions						TOTAL				
Rheumatoid Factor(s) (R. F.)		Antinuclear Factor(s) (A. N. F.)		Auto-immune complement fixation (A. I. C. F.)		Positive reactions	Number of tests		Subjects with one or more auto-antibodies	
No.	%	No.	%	No.	%	No.	No.	%	No.	%
0	0	0	0	0	0	0	238	0	0	0
0	0	0	0	0	0	4	700	0.57	4	4.00
3	1.93	0	0	4	2.58	19	1085	1.75	17	10.96
3	2.00	0	0	1	0.66	19	1050	1.80	19	12.66
5	4.58	0	0	2	1.83	25	763	3.27	23	21.10
14	11.96	2	1.70	2	1.70	42	819	5.12	39	33.33
7	12.50	2	3.57	0	0	28	392	7.14	23	41.07
7	8.43	1	1.20	0	0	51	581	8.77	36	43.37
39	*4.85*	*5*	*0.62*	*9*	*1.11*	*188*	*5628*	*3.34*	*161*	*20.00*
0	0	0	0	1	3.12	2	224	0.89	2	6.25
2	2.00	0	0	2	2.00	11	700	1.57	10	10.00
5	4.80	3	2.88	7	6.73	32	728	4.39	30	28.84
3	3.15	1	1.05	1	1.05	24	665	3.60	19	20.00
3	3.44	3	3.44	0	0	30	609	4.92	26	29.88
3	3.15	1	1.05	1	1.05	48	665	7.21	41	43.15
3	5.66	0	0	0	0	35	371	9.43	27	50.94
11	18.33	0	0	2	3.33	39	420	9.28	31	51.66
30	*4.79*	*8*	*1.27*	*14*	*2.23*	*221*	*4382*	*5.04*	*186*	*29.71*
69	**4.82**	*13*	**0.90**	*23*	**1.60**	*409*	*10010*	**4.08**	*347*	**24.20**

far below the values found in Hashimoto's disease, pernicious anaemia and rheumatoid arthritis, somewhat higher levels were occasionally found for antithyroglobulin (1/2,500), gastric antibodies (1/128), rheumatoid factor (1/2,500) and A. I. C. F. (1/256).

Of the 804 male subjects 161 (20.02 %) and of the 626 females 186 (29.71 %) had positive serological reactions. This *difference between the sexes* is statistically significant ($P < 0,001$). With the single exception of the rheumatoid factor, the incidence of individual antibodies was considerably higher in females. On the other hand the difference between the sexes is largely due to the difference in the incidence of thyroglobulin antibodies. This finding corresponds well with the clinical known fact that auto-immune thyroiditis, systemic lupus erythematosus, etc. have a higher incidence in women.

We found an *increase in the incidence* of circulating auto-antibodies *with advancing age*. If we divide our series in two groups one containing all subjects below the age of 50 and the other all those over 50 years, we find that in the group below 50,150 out of 966 (15.52 %) were positive, while in the second group 197 out of 464 (42.45 %) had positive reactions. The difference between these percentages is statistically significant. Also the difference in the percentage of positive reactions to single antibodies (except A. I. C. F. and A. N. F.) is statistically significant. The fact that this higher incidence concerns both the organ-specific and the non-organ-specific auto-antibodies suggests that this has nothing to do with changes in individual organs, but is rather an expression of an immunological disorder of old age which is difficult to interpret.

In order to find out whether any *correlations* exist between various individual auto-antibodies, we divided our positive cases into 3 groups:

1) Those that had thyroid antibodies (antithyroglobulin, microsomal fraction antibodies and anti-CA2);
2) Those that had gastric antibodies;
3) Those that had the rheumatoid factor.

Among them we looked for the presence of other organ-specific and non-organ-specific auto-antibodies and their incidence was compared with that of other cases of the same sex and age-group who had neither thyroid, gastric antibodies nor rheumatoid factor, cases we will call controls.

The results are shown in the tables II, III, IV. We found that:

a) In persons having thyroid auto-antibodies, the gastric antibodies were more frequently encountered: 12.20 % as compared with 6.10 % in the controls ($P < 0.05$). On the other hand the non-organ-specific antibodies were present either in equal or inferior numbers than in the controls.

b) Among those that had the gastric antibodies, thyroid auto-antibodies were also more frequent: 34.72 % as compared with 22.22 % in the controls ($P = 0.05$). Yet in these subjects the incidence of A. N. F. and A. I. C. F. was less frequent than in the controls, while there was no difference in the incidence of the rheumatoid factor.

c) In all cases that had a positive rheumatoid factor the incidence

TABLE II

Subjects with thyroid auto-antibodies

Comparison of the incidence of positive serological reactions to organ-especific and non-organ-especific auto-antibodies between two groups of healthy subjects with and without thyroid auto-antibodies.

	Number of Subjects	POSITIVE SEROLOGICAL REACTIONS							
		Gastric Antibodies		Rheumatoid Factor (s) (R. F.)		Antinuclear Factor (s) (A. N. F.)		Auto-Immune Complement Fixation (A. I. C. F.)	
		No.	%	No.	%	No.	%	No.	%
Subjects with thyroid auto-antibodies	213	26	12.20	11	5.16	1	0.46	4	1.87
Subjects without thyroid autoantibodies	213	13	6.10	18	8.45	2	0.93	4	1.87
		$X^2 = 4.169$ $P < 0.05$		$X^2 = 1.810$ N. S. D.		$X^2 = 0.334$ N. S. D.		— —	

N. S. D. = No Significant Difference.

TABLE III

Subjects with gastric antibodies

Comparison of the incidence of positive serological reactions to organ-especific and non-organ-especific auto-antibodies between two groups of healthy subjects with and without gastric antibodies.

	Number of Subjects	POSITIVE SEROLOGICAL REACTIONS							
		Thyroid auto-antibodies		Rheumatoid Factor (R. F.)		Antinuclear Factor (s) (A. N. F.)		Auto-Immune Complement Fixation (A. I. C. F.)	
		No.	%	No.	%	No.	%	No.	%
Subjects with gastric antibodies	72	25	**34.72**	7	**9.72**	0	**0**	0	**0**
Subjects without gastric antibodies	144	32	**22.22**	14	**9.72**	2	**1.38**	2	**2.89**
		$X^2 = 3.841$ P = 0.05		— —		$X^2 = 1.007$ N. S. D.		$X^2 = 1.007$ N. S. D.	

N. S. D. = No Significant Difference.

TABLE IV

Subjects with rheumatoid factor

Comparison of the incidence of positive serological reactions to organ-especific and non-organ-especific auto-antibodies between two groups of healthy subjects with and without gastric antibodies.

	Number of Subjects	POSITIVE SEROLOGICAL REACTIONS							
		Thyroid auto-antibodies		Gastric antibodies		Antinuclear Factor (s) (A. N. F.)		Auto-Immune Complement Fixation (A. I. C. F.)	
		No.	%	No.	%	No.	%	No.	%
Subjects with rheumatoid factor	69	10	14.49	8	11.59	3	4.34	0	0
Subjects without rheumatoid factor	138	30	21.73	10	7.24	1	0.72	4	2.89
		$X^2 = 0.422$ N. S. D.		$X^2 = 1.093$ N. S. D.		$X^2 = 3.187$ N. S. D.		$X^2 = 2.038$ N. S. D.	

N. S. D. = No Significant Difference.

of concomitant A. N. F. was increased to 4.34 % as against 0.72 % in the controls. There was however no difference in the incidence of organ-specific auto-antibodies.

The important correlation between thyroid and gastric auto-antibodies described by IRVINE ET AL. (1962) and by DONIACH ET AL. (1963) in patients suffering from Hashimoto's disease and pernicious anaemia is thus also demonstrable in our series. On the other hand there appears to be no correlation between organ-specific and non-organ-specific auto-antibodies.

These results confirm the distinction between *primary* auto-immunization directed towards normal generally distributed cells and circulating structures, attributable to a primary alteration of the immunological apparatus, and *secondary* auto-immunization due to lesions in specific organs, forming organ-specific auto-antibodies.

* * *

At the present state of our knowledge it is difficult to determine the *significance* of the presence of organ-specific and non-organ-specific auto-antibodies in clinically healthy persons. Three interpretations can be suggested as already mentioned. The first is that this is an expression of post-clinical auto-immunization which, as judged from our material, does not seem very likely, as none of the persons tested showed any evidence of ever having suffered from an auto-immune disease. The second is that we are dealing with an early, as yet asymptomatic or pre-clinical auto-immune disease. Generally speaking this possibility cannot be excluded, particularly as BALL and LAWRENCE (1961) have described in a few cases the presence of auto-antibodies prior to the onset of the disease. The third interpretation is that of a latent auto-immunization due to a constitutional predisposition to auto-immune phenomena. This is supported by the high incidence of auto-antibodies found among relatives of persons suffering from auto-immune disease. In this respect systemic lupus erythematosus has been studied by BRUNJES ET AL., MARLOW ET AL., BLUMENFELD ET AL. and others. This aspect of rheumatoid arthritis was investigated by TEODORI and NERI SERNERI, ZIFF, LAWRENCE and BALL and others as Hashimoto's disease by IRVINE ET AL., HALL ET AL., AUSTONI ET AL. and others.

Such investigations have led to the suggestion that in these diseases there exists an immunological abnormality which is genetically transmitted and that, as is often the case with hereditary diseases, the abnormality can express itself in varying degrees of intensity.

In the case of healthy persons our lack of knowledge of the exact mechanism whereby auto-antibodies are formed, makes it impossible to say why some have circulating auto-antibodies. For some auto-antibodies one might assume that there exists an alteration in the mechanism of immunological tolerance, while for others, particularly the organ-specific auto-antibodies, one can think of a state of immunological hyperreactivity towards "poor" antigens (WAKSMAN). In favour of such an hypothesis we can quote our finding that in 239 allergic patients who had

demonstrable skin-sensitizing antibodies 103 (43 %) also had thyroid auto-antibodies and 24 (10 %) gastric antibodies, while in 717 healthy persons of corresponding age and sex only 68 (9.4 %) and 27 (3.7 %) respectively were positive (SERAFINI ET AL., 1963, 1964).

The fact that we found such an unexpectedly high incidence of 24.26% of circulating auto-antibodies in healthy persons leads us to believe that this indicates the existence of a proliferative reaction of certain groups of immunologically competent cells. Yet this immunological abnormality persisting for many years and possibly for the duration of life, does not necessarily give rise to tissue lesions or disease. It simply shows the presence of a complex immunological defect which is probably of genetic origin and which might be called a state of *latent auto-immunization* or better perhaps *auto-immune diathesis*. There does however remain the possibility that in some individuals, in which a systemic or local alteration of the mechanisms of delayed sensitivity may be evoked by various factors such as infections, drug reactions etc, an auto-immune disease may develop, be it in the connective tissue or in specific organs.

Simultaneous testing for the most important auto-antibodies combined with further serological and genetic studies will not only reveal the presence of an auto-immune diathesis, but may, when associated with investigations on the reaction of such patients to administered antigens, prove to be a new method of investigating the mysteries of the auto-immune diseases.

If we presume that the presence of circulating auto-antibodies in healthy persons indicates a particular predisposition towards auto-immune disease, such investigations would have the further advantage of enabling us to take suitable prophylactic measures.

SUMMARY.

The authors briefly mention the significance of the presence of auto-antibodies both in disease and in healthy subjects.

Concerning the latter point, they refer to studies conducted on the incidence of some of the most significant organ-specific and non-organ-specific auto-antibodies in 1,430 subjects in apparent health, grouped according to sexes and ages. The following percentages were found: thyroglobulin antibodies 6.85 %; second colloid antigen (CA2) antibodies 5.66 %; thyroid microsomal fraction antibodies 3.70 %; gastric antibodies 5.03 %; rheumatoid factor 4.82 %; antinuclear factor(s) 0.90 %; auto-immune complement fixation 1.60 %. Among the subjects tested, one or more auto-antibodies were found in 347 (24.26 %).

The incidence of positive serological reactions was considerably more frequent among females and progressively increased with age in both sexes.

Regarding the organ-specific auto-antibodies, a serological correlation between thyroid and gastric auto-antibodies were found.

The authors suggest that a high percentage of circulating auto-antibodies in the normal population can be indicative of an immunolo-

gical defect, possibly of genetic origin, which might be called "latent auto-immunization" or better perhaps (auto-immune diathesis". This defect is not a sign of disease, but indicates a "potential" state of possible disease, which may be brought out by various trigger mechanisms.

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