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TISSUE REACTIONS TO PROTEIN SENSITIZATION*

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As this account is in no way intended to be exhaustive, but rather to illustrate the scientific basis of medicine in one field, it will not be out of place briefly to consider the nature of the processes by which science is built. They may be represented diagrammatically:

The starting-point has always been an observation, usually made in the midst of some practical performance—in medicine, when caring for a patient or examining his body after death. This clinical observation (often associated with others made previously) leads to the formulation of a hypothesis on how the events happened or what was the relation between one observation and another. In scientific medicine such hypotheses are tested by experiment, an attempt being made to reproduce the events observed at will or to replace them by some other event. The success or failure of these efforts leads on to fresh modified hypotheses—sometimes also modified by further observations—and so the cycle goes on. Increasingly, knowledge based on facts replaces speculation.

Much has been written about the nature both of observations and of experiments, good and bad, and there is still much to be learned about these aspects of scientific method. Rather less attention has been paid to the scientific hypothesis. Here only two points can be stressed. In the first place, every effort should be made to keep separate in one's mind the facts of observation and experiment from the theory or hypothesis. This is more easily said than done, for even the description of a fact may lead to hypothetical ideas slipped in with the very words used. Secondly, the distinguishing feature of a good hypothesis is its usefulness, perhaps even more than its ultimate truth. Useful hypotheses are precise and point to crucial experiments which can be performed with the resources available. The remark-

able success of most outstanding men of science—and clinical science is no exception—has been due in no small part to their flair for formulating the relevant and right hypothesis at the right time.

To illustrate this scientific basis of medicine in the field of protein sensitization we should try to proceed always from observations on man rather than on animals, and from the "known" (or relatively well understood) phenomena to the "unknown" (or more obscure). With this in mind, I propose to discuss two groups of reactions only, those of the *asthma-hay-fever-urticaria* type, and those showing reactions of the *tuberculin-trichophyton* type. Together they include a wide range of diseases and disease processes. They omit types of reaction of great interest, such as serum sickness or homograft reactions—although a good scientific basis has now been established for discussing the nature of these processes. It is also necessary to omit many disordered functions classified to-day as rheumatic fever, polyarteritis nodosa, etc.—a less serious omission, since we are still not on sure ground in regarding these as examples of protein sensitization reactions, however suggestive one may think the evidence.

Asthma-Hay-fever-Urticaria Group

The first clinical observations linking asthma with sensitization were made by Hyde Salter, of London, and published in a monograph in 1860, "animal emanations" being blamed for the sufferings of many of his patients. Hay-fever had been accurately described some 30 to 40 years previously. In 1873 Blackley, a Manchester practitioner, published his *Experimental Researches on the Causes and Nature of Catarrhus Aestivus (Hay-fever or Hay-asthma)*, a book of such clarity and method that it should be studied to-day by anyone intending to work in this field. Like many others who have investigated this group of disorders, Blackley himself suffered with severe hay-fever. By testing his own skin he showed that reactions could be induced by some of the agents suspected of eliciting the symptoms in his nose and bronchi. He was thus able to examine current hypotheses, such as the causative effects of dust as such. Road dust from various sources rubbed into scarified skin produced no reaction, but pollen of certain kinds resulted within a few minutes in the now familiar sequence of events, with local swelling as a prominent feature.

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Blackley's skin tests on himself might to-day be regarded as somewhat heroic in dosage, for he describes reactions lasting 24 hours or more, equalling an egg in size; at all events, he obtained results differing considerably from his controls. He was able in this way to define two basic facts about the tissue reactions to protein sensitization of the hay-fever type: (1) the reactions could be reproduced from a number of tissues—the skin of many areas, the conjunctiva, the linings of the nose and bronchi; (2) the reactions were narrowly specific. In his own case strongly positive results were given by the pollens of grasses and of another monocotyledon, the iris. Negative results were obtained with pollens from other (dicotyledonous) flowers. Even more striking examples of specificity have been given later, by examples such as that of a child sensitive to horses but able to ride a donkey in comfort. Blackley's hypotheses were naturally not all correct: obsessed somewhat with his own observations on grass pollens, he speculated about those patients suffering with asthma from animal emanations, pointing out that most of the animals concerned tended to be closely connected with hay—the kind of "association" pitfall which no doubt is trapping all of us to-day in other clinical ideas.

Nature of Skin Reactions

The nature of the skin reactions to protein substances causing asthma and hay-fever is now comparatively well understood. The local reddening and oedema with the surrounding flare—the triple response—were analysed by Sir Thomas Lewis (1927) in a familiar series of experiments of great beauty. The use of histamine to excite closely similar reactions led to the idea of a local release of a diffusible poison or H-substance, with effects limited to the blood vessels. Close comparison of the response to histamine and to protein antigens in suitable subjects by Hare (1926) showed that the two reactions were practically indistinguishable except perhaps for slight delay at the protein site. Similar local oedema and reddening occur in the conjunctiva and mucous membrane of the nose. By analogy with animal anaphylaxis, smooth-muscle contraction has been considered as an additional basis for the reactions of the bronchi in asthma, although it is not known certainly if this occurs in man.* d'Abreu (1940) has described an asthmatic reaction viewed through the bronchoscope: certainly, the reduction of the bronchial lumen was rapid and intense.

The local outpouring of secretions from nasal and bronchial mucous membrane in asthmatic and hay-fever attacks is not fully understood in terms of H-substance reactions. The two salient features of this type of reaction in any tissue are the rapid onset of oedema—within five minutes—and the minimal changes detectable by histological methods. Necrosis, for example, is never found even after considerable local dosage of sensitizing protein. Cellular infiltration, except on occasion for some local eosinophilia, is likewise absent.

The general rule that scientific observations and experiments in any field, initially qualitative, are found to be more rewarding when brought on to a quantitative basis can be illustrated here. If the ordinary prick test is performed with histamine as a test substance the errors of the method and the dose-response relationship can be worked out (Squire, 1950). Modern statisti-

cal techniques lighten the labour and extract the maximum information from the results. The prick test, carefully performed, can be shown to have a coefficient of variation (an "error") of not more than 20% for dosages resulting in wheal sizes of 1–6 mm. diameter, provided that standard and comparison tests are made on symmetrically placed sites, left and right, and within a short time of one another. Further errors are introduced if comparisons without standards are made on different days or on different sites (even, say, a few inches further down a forearm).

The diameter of the wheal responses is roughly proportional to the logarithm of the concentration of histamine (or of protein in a sensitive subject). In other words, if the wheal diameter is increased 1–1.5 mm. by raising the strength of the solution from 1:100,000 to 1:10,000 a similar further increase in size will be found by using a solution 10 times stronger still. The amount of solution introduced by a standard "prick"—forcible enough to cause momentary pain, but insufficient to draw blood—can be shown to be about 3 millionths of a millilitre.

From such quantitative considerations it can be shown that tissue reactions are produced by very small amounts of protein in asthmatic patients. A wheal 3 mm. across can be produced, for example, by as little as 10^{-13} g. of horse dandruff protein (probably as little as 10,000 molecules of protein). Similar responses in such a patient were only obtained with histamine solutions weight for weight 1,000 times stronger. As the molecular weight of the dandruff protein is probably 1,000 times as great as that of histamine, we must suppose (if histamine is indeed the reaction-mediating H-substance) that each molecule of dandruff protein introduced into the skin of certain asthmatics liberates (and that within half a minute) about one million molecules of histamine. This hypothesis perhaps needs re-examination. At all events, quantitative skin testing, with standard histamine to check the skin's reactivity and saline controls to avoid errors due to dermatographia, as well as with test protein solutions of known concentration suspected to be responsible for protein sensitization in any given patient, is still a rewarding field for further experiment.

Transfer of Sensitivity

Apart from specific reactions to certain foreign proteins widely disseminated through the body, the tissues of asthmatic patients show no obvious abnormality. Their skins are not, for example, in any way abnormally sensitive to histamine or to other known pharmacologically active bases. The serum or plasma of such patients is abnormal, however, in that, transferred to the body of a normal person, it induces exactly similar specific sensitivity. Clinical observations again gave the lead when Ramirez (1919) observed that blood transfusion from an asthmatic donor resulted in a sensitized recipient. Loveless (1941) made this the basis of experimental observations, showing that the induced sensitivity affected skin, conjunctiva, and mucous membranes, being fully established within 24 hours and lasting, though waning in degree, over periods as long as 30 days.

The local transfer of sensitivity to a small area of normal skin by means of an intradermal injection of, say, 0.05 ml. of asthmatic serum (Prausnitz-Küstner technique) has been widely used and is of practical and theoretical importance. The "antibody" from the

*But see Schild, H. O., Hawkins, D. F., Mongar, J. L., and Herxheimer, H., *Lancet*, 1951, 2, 376.

patient is to some extent "fixed" locally, for such a sensitized site is capable of reacting, even if testing is deferred for several days. In particular, this kind of experiment can serve as the basis of a quantitative assessment of sensitizing antibody circulating in the plasma of an asthmatic or hay-fever patient. For a sensitized site, having once given a full reaction, is "discharged" and cannot again be caused to react, even with much stronger test doses of sensitizing protein. This discharging reaction can be obtained either by pricking the sensitized site after, say, 24 hours, or by mixing sensitizing proteins in graded dilutions with the asthmatic serum before introducing it to the normal skin test sites.

If the minimal quantity of sensitizing protein required fully to discharge a site treated with a known quantity of asthmatic serum is regarded as an "equivalence dose" at least, comparative indications are obtained of the amounts of sensitizing antibody per millilitre of any patient's serum. Most immunological reactions—for example, precipitin reactions—involve not very dissimilar numbers of molecules of antigen and antibody. If these passive transfer reactions are assumed to follow a similar pattern, then it seems that quite small amounts of sensitizing antibody—for example, less than 1 mg./ml. of serum—are involved even in highly sensitized asthmatic patients.

Generalizations on Foreign Proteins

Turning to the foreign proteins responsible for tissue reactions in sensitization of the asthma-hay-fever type, certain tentative generalizations are now possible. Those adequately studied—for example, the proteins in grass pollen (Harley, 1937), ragweed pollen (Stull *et al.*, 1931), house-dust (Rimington *et al.*, 1947), and horse dandruff (Squire, 1950)—are all of the general nature of albumins. They are digested by pepsin in acid solution and lose their power to provoke reactions. They are precipitated from solution by saturation with ammonium sulphate, but not by half-saturation. My colleague, Mr. D. R. Stanworth, has been studying horse-dandruff albumin in parallel with recrystallized horse-serum albumin (both of these proteins often giving reactions in patients and

suitably immunized animals). The electrophoretic mobility of the main component of the unpurified dandruff albumin closely resembles that of serum albumin in barbitone buffer (see illustration). This general similarity between the proteins sensitizing man by inhalation as dusts is perhaps not extraordinary, being related probably to their molecular size. In general, very small molecules are not potent antigens, while large protein molecules withstand drying poorly, usually being denatured and rendered insoluble. Perhaps, too, large molecules would fail to penetrate the mucous membranes, and so never have an opportunity to sensitize anyone. Guesses of this kind may indicate why the proteins concerned are all found among the "medium-sized" proteins. There is no evidence that they are otherwise related, and they seem to vary considerably in chemical composition—for example, in combined carbohydrate content.

The proteins known to cause sensitization in man are fully antigenic in animals. Precipitins are formed in response to injection, and animals such as guinea-pigs become sensitive, developing anaphylaxis when re-exposed after suitable intervals. Sensitization and shock can be induced, as shown by Ratner (1939), by letting animals inhale a fine spray of dust of such proteins. Sensitization in man can be assumed to follow absorption of the proteins either by inhalation or by ingestion. Direct evidence is lacking, but previous exposure as a cause is strongly suggested by the absence of specific sensitivity (even when sought) in areas of the world which are free from some special protein—for example, that of ragweed pollen in Europe (see Cooke and Sherman, 1950). Normal persons can absorb proteins through the gut, intact enough at least to excite reactions in passively sensitized sites. Lewis used to describe an experiment which failed—but succeeded in a different sense. He had sensitized a site on his forearm with some serum from a fish-sensitive subject, intending to test some preparations of protein by pricking them into the site next day. On leaving his home in the morning, however, he felt his forearm itching, and realized that a reaction had occurred

Comparison between electrophoretic mobilities of horse-serum albumin and horse-dandruff proteins. (Barbitone buffer pH 8.6, $\tau/2 = 0.1$.) Note that main component of dandruff resembles serum albumin, but that a minor component of greater mobility is also present in dandruff. (Electrophoresis conducted by Mr. D. R. Stanworth.)

prematurely. Unthinkingly, Lewis had accepted and eaten a kipper served for his breakfast (see Lewis and Grant, 1926).

The riddle of asthma, then, is not so much, How can a patient have been sensitized? as, Why are we not all sensitized? Our food, the very air we breathe, is charged with proteins capable of sensitization, and normal individuals absorb these proteins, at least to some extent. True, hay-fever of mild degree is a very common disorder, but well-marked asthma due to protein sensitization in otherwise healthy young persons is not so common. The riddle of the familial tendency to develop sensitization of this degree is still unsolved. Perhaps it implies that intact proteins are more freely absorbed by subjects who are liable to sensitization, though so far no evidence in favour of this idea has been presented. The careful quantitative studies now being conducted in animals to establish the dosage-response relationships between foreign proteins and anaphylactic sensitivity (Kabat, 1947; Coulson and Stevens, 1949) may offer fresh clues and suggest new experiments to be conducted on suitable patients.

Blood Eosinophilia

A feature commonly found in patients of the asthma-hay-fever-urticaria group is a blood eosinophilia. Repeated injections of protein, though each may be temporarily followed by a drop in blood eosinophils, induces eosinophilia in animals (Biggart, 1932). Many observations, but comparatively few experiments, are reported which could help to establish the significance and function of the eosinophil cell. The asthmatic patient's sternal marrow may show slight evidence of increased eosinophil production. If such a patient is kept free from exposure to the proteins to which he is sensitive (for example, in hospital) a gradual decline, say from 600-700 to 300 eosinophils per c.mm., over a period of about six weeks occurs (unpublished observations on a single patient). Such studies have been made reasonably accurate by the direct counting methods of eosinophil cells (Discombe, 1946).

In man and in animals, sensitized tissues affected by exposure to the appropriate foreign proteins may become crowded with eosinophil cells some hours later. As a working hypothesis, the eosinophil cell may be regarded as being concerned with "mopping-up" the products of tissue damage. Certainly, the large granules, so refractile under dark-ground illumination, so dense under phase-contrast microscopy, have some very specific function. In thin films of blood examined when warm and fresh the eosinophil is just about as active as a neutrophil polymorph leucocyte, moving with all its granules (except one or two) foremost. Phagocytosis of cocci by eosinophils is not readily observed in contrast with the behaviour of the polymorph. But the eosinophils will adhere to wettable surfaces, and on occasion—when offered, for instance, clumps of dead tubercle bacilli—they will lie close with only the granules in movement, circulating internally as if allowing different granules a period of active absorption close to the cell surface followed by a period of recovery deep in the cell body.

Are the granules acid (and avid for eosin) and capable of adsorbing base? Apparently, disruption of the eosinophil is the main source of histamine from the cells of the blood (Code, 1937). We are close to confusing fact and hypothesis in this account, but at least it can be said that the eosinophil cell is worthy of

greater attention. Easily distinguished and handled as a living cell, its granules appear intact if the cell is disrupted by pressure—they could be separated and analysed.

Tuberculin-Trichophyton Group

The reactions to tuberculin have occupied the attention of physicians ever since Koch (1890) made the unfortunate claim for this substance as a remedy for tuberculosis. It is now clear that sensitization closely analogous to that which follows infection with tubercle bacilli may occur with other infections—for example, by species of the fungus *Trichophyton*, as in athlete's foot. As the details of tuberculin sensitization have been more fully studied, it seems best to concentrate upon these reactions which serve as a type for the whole group. Much space in Arnold Rich's (1944) absorbing book is given over to describing the facts of sensitization. Here only the salient features can be mentioned, with particular stress on the similarities and differences between asthma-type and tuberculin-type sensitizations.

The quality of specificity of the tuberculin reaction is just as striking as that of the asthma-hay-fever group. In fact, the practical application of this form of sensitization at present is in diagnostic tests, which determine whether or not an individual has been infected with tuberculosis. Just as in hay-fever the skin can be shown to be sensitive to many species of grass, so in patients infected with either human or bovine strains of bacilli skin reactions to any tuberculin can be demonstrated. Unrelated infections, however, as with *Trichophyton*, confer sensitization specifically, to, say, trichophyton, and not at all to tuberculin.

Sensitization of the tuberculin type can be shown to be widespread throughout the body, to a greater extent even than in asthma-type sensitization. Lung, kidney, testes, meninges, cornea—all show evidence of sensitization when tested experimentally (see Rich, 1944). The skin has been studied most, as it is most easily observed. When compared with the wheal-reaction characteristic of asthma-type skin tests, the tuberculin reaction differs profoundly even on naked-eye observation. Most striking is the difference in time relations. The mild asthma-type reaction appears in a matter of minutes and fades in half an hour. The tuberculin reaction may just be evident some eight hours after the introduction of the test solution, is at its height after 24 hours, and disappears only after some two to three days. Even then signs of the reaction may persist in the form of slight pigmentation and some flaking of the epidermis, for lasting cytological changes are present in the dermis. Overdosage with tuberculin causes local necrosis, never seen with the skin tests for asthma. Systemic reactions, too, may occur, but again these differ from those following overdosage with the agents which elicit asthma and urticaria, by being slower in onset and more long-lasting, with fever and a general bodily disturbance.

Dosage-response relationships have been worked out with tuberculin as with asthma-type reactions (Wadley, 1949; Long and Miles, 1950). A convenient index of reaction strength is again the diameter of the local oedematous area, but in this case measured after 24 hours rather than after 10-15 minutes. The diameter of the local lesion is also proportional over a fair range to the logarithm of the dose of tuberculin introduced.

Histological Changes

The histological changes accompanying the tuberculin reaction have been described and debated in detail. There can be no doubt about the striking degree of these changes, in contrast with the asthma-type reactions, nor about the extensive cellular infiltration with the various types of wandering cell, including polymorphs and mononuclears, as seen in many kinds of inflammation. Histological examination of lesions is in this case more delicate than clinical observation, in the sense that changes can be seen in sections of skin removed from tuberculin-test sites before the reactions are visible to the naked eye.

The observation that wandering cells participate in these reactions falls into line with the later experimental findings that tuberculin sensitivity can be passively transferred from one individual to another, not as in the Prausnitz-Küstner technique by serum, but only by the transfer of cells (leucocytes). This striking advance was achieved by Chase in 1945. Guinea-pigs were artificially sensitized by injection of tubercle bacilli. When concentrated suspensions of their leucocytes (0.2 ml.) were introduced into normal animals by intravenous or intraperitoneal injection the recipients were clearly sensitized as judged by tuberculin skin-testing, albeit for a few days only. These experiments have been amply confirmed by other workers in the United States, and extended by the work of Lawrence (1949), who showed that more striking degrees of sensitization can be induced in man. Lawrence used relatively smaller dosages of leucocytes, but the human cell donors were probably more highly sensitive than Chase's animals, being selected from patients naturally infected with tuberculosis. The tuberculin-negative recipients were injected intradermally. Sensitization then spread in many instances to involve even remote skin sites, and lasted sometimes for a month or two as judged by the usual intradermal tuberculin tests.

The importance of the motile cells in tuberculin sensitization is also underlined by tissue-culture experiments. The experiments of Rich and Lewis reported in 1932 can justly be described as classical, if not on grounds of antiquity then on the basis of their elegance. In suitable concentrations tuberculin gave no appreciable effect when added to cells grown from tissues of normal guinea-pigs, even if serum from sensitive animals was also added. But cells from guinea-pigs previously sensitized, even when washed and cultured in normal guinea-pig serum, were damaged by similar concentrations of tuberculin. Fibroblasts perhaps were relatively unharmed, but wandering mononuclears migrating out in tissue culture were slowed down, stopped, and eventually killed. Recently, Cruickshank (1951) has extended the idea that not all types of cell from sensitized animals are susceptible to tuberculin, by showing that sensitized guinea-pig epidermis can grow in media containing concentrations of tuberculin considerably above those required to excite gross reactions in the skin of the intact donor animal.

The generalization that tuberculin sensitivity is not dependent upon a serum factor is not disturbed by the experiments of Miller and Favour (1951). These workers have demonstrated that further complexities may be found in the serum of patients in certain stages of tuberculosis, as judged by acute behaviour changes in various blood leucocytes when acted upon by serum-tuberculin mixtures (Favour *et al.*, 1949).

Whereas a special study of the eosinophil leucocyte is needed to increase our knowledge of the asthma-urticaria type of sensitization, with regard to the tuberculin-trichophytin reactions the focus narrows on to the lymphocyte—or at least to the various types of mononuclear cell. These cells are very active in tissues, wandering rapidly from site to site, as described by Ebert *et al.* (1940) in the rabbit's ear.

The chemical eliciting the tuberculin reaction is undoubtedly protein in nature. The work of Seibert (1944) and others has shown that activity can be demonstrated in proteins of various sizes, but that the most highly active material, now usually known as P.P.D. (purified protein derivative) has a molecular weight of about 35,000, and is homogeneous in electrophoretic experiments. The material of lower molecular weight found in old tuberculin is presumably derived from P.P.D. by breakdown. But none of these protein fractions, injected alone by any route, has been shown to cause the development of tuberculin sensitization—another point of major difference from the anaphylactic form of sensitization. But even dead tubercle bacilli, injected in sufficient quantities, readily induce sensitization in suitable animals, such as rabbits and guinea-pigs. It has now been shown that the essential difference between the bacillus and the protein derived from it depends upon the presence of specific materials (perhaps capable of inducing phagocytosis by mononuclear cells), since suitable mixtures of tuberculoprotein with the wax of the tubercle bacillus injected into guinea-pigs have proved capable of inducing specific tuberculin-type sensitivity (see Raffel, 1948). Much remains to be discovered about the differing forms and degrees of sensitization which follow inoculations by different routes and in different mixtures with poorly soluble materials, though striking progress has recently been made, most clearly described (at the same time with a suitable sense of excitement) by Burnet and Fenner (1949).

Control of Sensitivity Reactions

The medical scientist is sometimes, not unjustly, accused of being less interested in the treatment of disease than in the original condition and its basis. This should not be so; not only does the understanding of disease mechanisms suggest rational lines of treatment, but the success or failure of therapy, rational or empirical, may often be used to validate or disprove current theories about the nature of a disease. From this point of view, three methods now in use for controlling the reactions to sensitization merit discussion—"desensitization," the "antihistamine" drugs, and cortisone.

Desensitization

This term was used to describe the effects of sublethal anaphylactic shock in experimental animals. After administration of suitable doses of specific protein, further injections may be without effect or may produce only minor reactions. This state of reduced reactivity, however, lasts only a few days at most; the only parallel in human medicine is probably found in such circumstances as when a horse-sensitive subject is treated with increasing doses of horse serum (starting with very small amounts) during a period of hours, so as to enable an adequate dose of some vitally needed antiserum to be administered. As in the animal, this desensitization is of limited duration.

On the other hand, a course of injections of, say, pollen extracts given to a patient regularly attacked by summer hay-fever, in increasing dosage at intervals of three to seven days, is well known to induce in a fair proportion of instances a gradual lessening of reactivity of skin and other sites to pollen tests and of the symptoms and signs of attacks. Several months of treatment are usually required, and the beneficial results may not last for more than one season.

The confusion of thought resulting from the use of the same word "desensitization" to describe these events has been unfortunate. The procedure required clearly suggests that some form of immunization process is involved, and experiments with the serum from patients treated in this way has confirmed the presence of a neutralizing antibody capable of combining specifically with the pollen protein in such a way that it no longer elicits skin reactions (Loveless, 1940; Harley, 1942). Simple tests—for example, stability to heat—can be used to differentiate this neutralizing antibody from the sensitizing substance originally present in the hay-fever subject's serum. These important findings deserve further work, directed, in particular, towards easier methods of determining *in vitro* the concentration of neutralizing antibody achieved at various stages of this form of treatment. The gradual reduction of tuberculin sensitivity which can be achieved by successive injections of the appropriate protein has not, apparently, been tested to determine whether a similar or different mechanism is involved.

Antihistamine Drugs

These were originally discovered by Bovet and Staub (1937) in Paris. Halpern (1950) has reviewed his own and other contributions to the introduction of compounds suitable for human administration, and has summarized recent experimental work. The antihistamine drugs are usually strikingly effective in controlling urticaria and hay-fever. Caution must be exercised, however, in drawing conclusions from this finding, since only some of these drugs single out histamine for antagonism (see Gaddum, 1948). Quantitative skin-testing shows that a great reduction occurs in the wheal-like responses to specific protein inoculation. Guinea-pig lung reactions to histamine, and (in sensitized animals) to doses of protein considerably greater than those known to cause death in unprotected animals, are abolished. In man, control of asthma is less certain; in some trials the patients' reports of improvement may have been vitiated by insufficient control of the "suggestion" factor—for example, failure to use dummy tablets—in others, the selection of patients has not been such as to ensure that true examples of sensitization were being studied. Whereas the asthma-urticaria group of patients are usually benefited by treatment with the antihistamine drugs, the sensitivity reactions of the tuberculin-trichophyton group are unaffected.

Cortisone

The effects of cortisone and of cortisone-liberating A.C.T.H. have been studied for too short a time for the formulation of more than tentative conclusions. It seems generally agreed at present that these drugs greatly diminish the naked-eye reactions to tuberculin, but have negligible effect on the wheal-like reactions to histamine or to agents such as pollen concerned with the asthma-urticaria type of response. It is clearly of very great importance to study the nature of this inhibition of

the tuberculin reaction. A local effect of cortisone on ground-substance of subcutaneous tissue can be demonstrated by measuring dye-spreading round the site of injection (Gell and Hinde, personal communication). Histological studies of tuberculin reactions at various stages in their development in the rabbit have shown a highly significant reduction in the proportion of mononuclear cells as compared with polymorphonuclear leucocytes (Gell and Hinde). Whether these and the various other known effects of these hormones can eventually be correlated into a simple picture is uncertain at present: in their effects on the tuberculin reaction, at least, it seems that once again the behaviour of the mononuclear wandering cell is of major importance.

Conclusions

The study of sensitization often seems to raise as many questions as answers—a sign, perhaps, of a field of investigation worthy of further cultivation. In the introduction to this paper the importance of hypotheses in the advancement of medical science was explained, with the suggestion that a sign of merit was current usefulness rather than a pretence of final truth. With this justification an outline may be given of some working hypotheses designed to stimulate experiments on sensitization.

The asthma-urticaria type of reaction is evoked in certain tissues by the combination of a specific antibody, which is derived from the patient's plasma globulin, with appropriate antigens. These antigens are proteins encountered in the natural environment, are of medium molecular size, are usually relatively stable to drying, and have presumably induced sensitization on some previous occasions when inhaled or ingested by the patient. The reactions in various tissues responsible for the symptoms and signs are mediated by the release of pharmacologically active substances, among which a base having histamine-like properties is prominent; subsequent neutralization of reaction products may be carried out by eosinophil leucocytes. Parenteral administration of the same antigen (though usually in amounts differing considerably from those absorbed under natural circumstances) can give rise to the formation of neutralizing antibodies which may reduce the degree of reaction to subsequent exposure.

Tuberculin-trichophyton type sensitization follows phagocytosis of certain forms of poorly soluble material—for example, wax—along with specific proteins derived from various micro-organisms. These phagocytes and similar wandering cells may then carry sensitivity widely through the body. On subsequent contact with specific protein—for example, tuberculin—these mononuclear cells react by releasing substances capable of dilating small blood vessels and attracting other mononuclear cells to the vicinity. Enzyme liberation may also occur, perhaps tending to digest the foreign protein and even, in sufficient concentrations, to affect also the local tissues and ground-substance. The functional activity of these mononuclear cells is controlled by circulating hormones—for example, cortisone and thyroxine.

Caution would have dictated the use of "perhaps" many times in these two paragraphs. The techniques, however, for proof or disproof are ready to hand, and hypotheses should not expect a long life. Heparinized blood can be separated by red-cell aggregation with dextran or fibrinogen; the supernatant, rich in white

cells, can be poured through glass wool, to which the polymorphs adhere. The filtrate has only to be lightly centrifuged to obtain lymphocytes—alive if suitably handled. Do these cells carry the essential properties required by the hypotheses? If so, under what circumstances? Similarly, many more experiments need to be performed before the explanation of the various types of “antibody” found in asthma can be regarded as “being on a scientific basis.”

Summary

Protein sensitization in man is a suitable subject for scientific study, especially when reinforced by animal experiments. Two main types—the asthma-urticaria and the tuberculin-trichophyton type—are considered and contrasted. Some of the methods of controlling sensitization reactions are discussed. Tentative hypotheses suggesting further experimental work are outlined.

REFERENCES

- Biggart, J. H. (1932). *J. Path. Bact.*, **35**, 799.
 Blackley, C. H. (1873). *Experimental Researches on the Causes and Nature of Catarrhus Aestivus (Hay-fever or Hay-asthma)*. London.
 Bovet, D., and Staub, A. M. (1937). *C.R. Soc. Biol., Paris*, **124**, 547.
 Burnet, F. M., and Fenner, F. (1949). *The Production of Antibodies*, 2nd ed. Melbourne.
 Chase, M. W. (1945). *Proc. Soc. exp. Biol., N.Y.*, **59**, 134.
 Code, C. F. (1937). *J. Physiol.*, **90**, 485.
 Cooke, R. A., and Sherman, W. B. (1950). In *Pathologic Physiology*, edited by W. A. Sodeman, p. 706. London.
 Coulson, E. J., and Stevens, H. (1949). *J. Immunol.*, **61**, 119.
 Cruickshank, C. N. D. (1951). *Nature, Lond.*, **168**, 206.
 d'Abreu, A. L. (1940). *Lancet*, **2**, 421.
 Discombe, G. (1946). *Ibid.*, **1**, 195.
 Ebert, R. H., Sanders, A. G., and Florey, H. W. (1940). *Brit. J. exp. Path.*, **21**, 212.
 Favour, C. B., Fremont-Smith, P., and Miller, J. M. (1949). *Amer. Rev. Tuberc.*, **60**, 212.
 Gaddum, J. H. (1948). *British Medical Journal*, **1**, 867.
 Halpern, B. N. (1950). *Acta allerg., Kbh.*, **3**, Suppl. 1, 164.
 Hare, R. (1926). *Ibid.*, **13**, 227.
 Harley, D. (1937). *Brit. J. exp. Path.*, **18**, 469.
 — (1942). *Studies in Hay-fever and Asthma*. London.
 Kabat, E. A. (1947). *Amer. J. Med.*, **3**, 535.
 Koch, R. (1890). Quoted by Rich (1944).
 Lawrence, H. S. (1949). *Proc. Soc. exp. Biol., N.Y.*, **71**, 516.
 Lewis, T. (1927). *The Blood Vessels of the Human Skin and Their Responses*. London.
 — and Grant, R. T. (1926). *Heart*, **13**, 219.
 Long, D. A., and Miles, A. A. (1950). *Lancet*, **1**, 492.
 Loveless, M. H. (1940). *J. Immunol.*, **38**, 25.
 — (1941). *Ibid.*, **41**, 15.
 Miller, J. M., and Favour, C. B. (1951). *J. exp. Med.*, **93**, 1.
 Raffel, S. (1948). *J. infect. Dis.*, **82**, 267.
 Ramirez, M. A. (1919). *J. Amer. med. Ass.*, **73**, 984.
 Ratner, B. (1939). *Amer. J. Dis. Child.*, **58**, 699.
 Rich, A. R. (1944). *The Pathogenesis of Tuberculosis*. Springfield, Illinois.
 — and Lewis, M. R. (1932). *Bull. Johns Hopk. Hosp.*, **50**, 115.
 Rimington, C., Stillwell, D. E., and Maunsell, K. (1947). *Brit. J. exp. Path.*, **28**, 309.
 Salter, H. Hyde (1860). *On Asthma: Its Pathology and Treatment*. London.
 Seibert, F. B. (1944). *Chem. Rev.*, **34**, 107.
 Squire, J. R. (1950). *Clin. Sci.*, **9**, 127.
 Stull, A., Cooke, R. A., and Chobot, R. (1931). *J. biol. Chem.*, **92**, 569.
 Wadley, F. M. (1949). *Amer. Rev. Tuberc.*, **60**, 131.

The annual report of the honorary secretary of the Joint Tuberculosis Council briefly records the work of the council during the year. Probably the most important achievement was the completion and publication of forms of record for use in chest clinics. At the request of the Ministry of Health the council drew up a memorandum on the internal administration of hospitals, and members of the council also assisted the Ministry in drawing up a report on skiagraphic terminology and radiographic technique. A trial of a four-digit classification of tuberculosis was undertaken at the request of the World Health Organization.

DENTAL DISEASE AMONG 14-YEAR-OLD LONDON SCHOOL-CHILDREN IN 1947 AND 1950

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The Education Act of 1944, which raised the school-leaving age to 15, gave the opportunity for making dental surveys of the 14-year-old group. This age is suitable for the examination of the permanent teeth, as most types have erupted, with the exception of the third molars, while few deciduous teeth remain to be shed. It also provides an overall picture of the state of dental fitness of a section of children the majority of whom are about to leave school, and as such it will no doubt be of interest to the public health authorities.

The two surveys with which this paper is concerned were carried out during the winter months of 1947–8 and 1950–1, but for the sake of brevity they will be referred to as the 1947 and 1950 surveys. The London County Council very kindly afforded facilities for making the inspections, all of which took place in schools under their authority, the number of children examined being 1,216 in the earlier year and 1,089 in the later. These children were unselected samples distributed between modern, technical, and grammar secondary schools, the proportions from each bearing a rough relation to that of the total child population attending the different kinds of secondary schools in the area. In both surveys approximately 72% were from the modern schools, 12% from technical schools, and 16% from grammar schools. Since more children attend modern schools, any differences between the teeth of these children in the two surveys have more influence on the total figures than have the differences between the teeth of technical or grammar pupils. The samples of children from the latter kinds of school were too small to give more than an indication of possible differences between them and the moderns, especially where individual types of teeth are concerned.

The examination procedure and the charts used for recording the dental condition of the children were basically the same as those employed in the surveys of 5-year-olds from 1943 onwards (see Mellanby and Coumoulos, 1946), but certain adaptations were made to suit the charts for use with the permanent dentition (see Chart). Although external structure (as gauged by M. Mellanby's probe method), the incidence and extent of caries, mottling of the enamel, tartar, gingivitis, alignment, and occlusion were recorded, in the present paper only the results relating to the incidence and extent of caries, gingivitis, and tartar are given.

Methods

Each child's mouth was carefully examined by one of us with a standard probe and illuminated mirror, the results for each individual being separately charted.