THE BIOCHEMISTRY OF IMMUNITY REACTIONS.*

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THE chemistry of immunity reactions has been actively investigated and the results constitute an extensive literature. The stage of simplification has not yet been reached, and later work has almost always added to the complexity of the subject.

Few now doubt the chemical basis of the alterations in the tissues and body fluids which constitute acquired immunity. To speak of immunity reactions, however, may be misleading, and it is more accurate to designate them antibody reactions, since the very opposite of immunity, anaphylaxis, may result from so-called procedures of immunization. The manifestations of antibody action are in most cases not peculiar, but consist in physical or chemical alterations which can usually be produced in various other ways. Thus absorption of complement is not effected only by an antigen-antibody complex.

In general, the characteristic feature of an antibody reaction consists in its being brought about by an antiserum, which, apparently, differs from other serums only in one constant feature—namely, its capacity to cause certain changes when associated with the homologous antigen. It is extremely difficult to classify antigens except according to the effects which follow when they are associated with the corresponding antibodies.

Thus it is possible to differentiate (1) antitoxic and anti-ferment action, (2) agglutination and precipitation, (3) complement fixation, which may or may not be accompanied by cytolytic action, (4) bacteriotropic or opsonic effects; in addition to these there falls to be considered (5) decompositions due to the action of antagonistic ferments—that is to say, substances supposed to be of ferment character, which have the property of breaking down into chemically simpler bodies the compounds in response to which they have been developed. This last class of reactive phenomena has been brought into prominence by the recent investigations of Abderhalden and his co-workers.

In order to obtain a clearer insight into the processes, it is necessary to consider (a) the constitution of antigens and the nature of the changes which they excite in the animal body, thereby leading to the production of antistances, and (b) the phenomena presented by the interaction of the constituents concerned in antibody reactions.

The Nature of Antigens.

The common type of antigen is a foreign protein, and it is immaterial whether it is derived from higher animals and plants or from bacteria or protozoa. The two principal features of antigens are: (1) That they are substances foreign to the species of animal which reacts; and (2) that they are usually so intimately associated with protein that it is impossible to obtain antigens altogether free from protein reactions. The latter characteristic is not shared by all antigens, however, if we are to include those substances which, according to Abderhalden, lead to the development of antagonistic ferments. Thus, Abderhalden found, confirming and extending earlier observations of Weinland, that a comparatively simple substance, such as saccharose, when introduced into the tissues of dogs, might lead to the appearance of invertase in their serum. With the possible exception of the group of bodies which excite the production of antagonistic ferments and also of Ford's amanita-haemolysin and toxin, which he holds to be glucosides, antigenic properties appear to be indissolubly associated with proteins or closely allied substances. The evidence brought forward to show that any of the so-called lipid bodies, such as "lecithins," in the isolated state can act as antigens is scanty and unsatisfactory. Jobling and Bull found that a haemolytic immune serum, with or without complement, when brought into contact with the homologous blood corpuscles, caused an increase in acidity, which they attributed to the splitting-off of fatty acids from lipoids contained in the red cells. Non-homologous red cells did not yield so

much acid as the homologous corpuscles. It was concluded, however, that the action of the specific immune lipase was not essential for haemolysis, since an immune serum, deprived of its lipase, by heating to 70° C., still produced lysis along with complement. There is no evidence that immune body is actually a lipid (Meyer). Apart from the singular results of Kurt Meyer obtained with tapeworm lipoids (confirmed by Thiele and Embleton), it has not been found possible to produce complement-deviating or precipitating antibodies by injecting animals with lipoids (Fitzgerald and Leathes, Ritchie and Miller). Lipoids are present in a "masked" state in practically all tissues and body fluids, and since it is probably impossible to remove lipid substances completely without profoundly altering the proteins with which they are associated, it is therefore difficult to determine in how far their presence may affect the antigenic action of proteins. The weight of evidence, however, goes to prove that where the development of antibodies, in response to lipoids, has been claimed, the possible fallacy due to the entangling ("adsorption") of proteins has not been excluded. As is known from the experiments of Friedberger and Fröhner on the development of haemolysins, an extremely minute amount of protein may suffice to lead to the production of recognizable quantities of antistances, and in the work of Bang and Forssman, which originally brought into prominence the question whether lipoids could act as antigens, the immune body developed in response to "lipoid" constituents of red corpuscles was exceedingly feeble.

Turning now to the apparently essential element of foreignness in an antigen, it appears that certain infections may so alter the reacting power of the body or may produce such a change in certain tissues that, as a consequence, an individual may develop antibodies to his own cells. Thus the pathogenesis of paroxysmal haemoglobinuria is to be explained by the fact that certain syphilistics develop a lysis for their own blood corpuscles. It is not known whether this haemolysin is produced as a true antibody response to constituents of the red corpuscles or whether it is of "heterogenetic" origin. The question whether autolysins for other tissues may develop and lead to disease merits consideration.

It is not yet possible to arrive at any clear conception with regard to the means whereby antibody production is excited. Recent work on the digestion and resorption of proteins has shown that, in the adult at any rate, the intestinal epithelium rigorously protects against the entrance into the tissues from the alimentary canal of albuminous material with foreign specificity. The direct contact of foreign protein with the tissues, such as occurs in immunization or in bacterial infections, has therefore no parallel in normal processes of nutrition; accordingly, the reactive phenomena following such parenteral introduction of protein must be regarded as essentially pathological. It is noteworthy that a natural infection may differ markedly from artificial immunization as a means of stimulating antibody reaction; thus the antibodies developed in the course of an attack of typhoid fever are in general feeble as compared with those resulting from several injections of typhoid vaccine. This may partially explain the efficacy of vaccine treatment in such conditions.

Phenomena of the Interaction of Constituents concerned in Antibody Reactions.

Here we are hampered by the fact that antibodies have never been isolated in the pure state, and the same is true of most if not all antigens. Consequently, in an antibody reaction one observes effects brought about by the interaction of certain components in a highly complex and irresolvable mixture. The influence of physical and chemical changes on the course of the reaction can be examined and important results have been obtained, but the question usually remains undetermined as to whether an alteration in the essential components has led to any given change, or whether a modification of the medium is primarily responsible. Antibody reactions have been extensively explained by reference to physico-chemical and colloid phenomena. The reacting components appear either to be colloids or else to be markedly influenced by close association with such substances. Thus, they tend

* A paper read in opening a discussion in the Section of Pathology and Bacteriology at the Annual Meeting of the British Medical Association at Aberdeen.

to be fixed by bodies which act in virtue of their surface (adsorption); also, they exhibit frequently "zone-phenomena," a particular effect occurring only when the components are present in certain relative amounts, excess of one constituent or the other tending to inhibit the reaction. Adsorption, however, occurs in characteristic form under conditions which exclude the essential feature of antibody reactions, namely, specificity. It has been shown (*vide* von Szily, also Landsteiner and Prasek) that red blood corpuscles treated with osmic acid can remove from an antiserum the immune body which has been developed by injections of red blood cells of a different species, but this does not explain why a certain portion of the immune body can be absorbed by intact corpuscles only when these belong to the homologous species. Again, the fact that passage through a Berkefeld filter removes complement, but not immune body, from the serum (Muir and Browning, see also Schmidt²), does not explain why a protein or a suspension of red blood corpuscles should be able to absorb considerable quantities of complement only when the corresponding antibody is present. Specificity, then, is one of the chief problems of antibody reactions. Specificity for the species has long been known, but recent observations show that a much more restricted degree of specificity may be exhibited by antibodies.

Mackie has found that a powerful antiserum, which agglutinates the culture of *B. coli* by means of which it was developed, in a dilution of 1 in 30,000, is entirely without agglutinating action on numerous strains of organisms derived from a similar source, which appear identical when tested by all the other available criteria; and further, these strains do not absorb the agglutinins from dilute solutions. On the other hand, complement fixation, brought about under the influence of the same antiserum, shows that in addition to the marked specificity for the homologous strain, there is also a wide group specificity, which includes organisms differing markedly in other biological properties such as the power of fermenting lactose.

Experiments of this kind show that the determination of biological relationships by means of antisera may depend greatly on the particular test employed. Here also it is impossible to generalize from the behaviour of any one species of antigen, and a law which applies to the immunity reactions of a particular group of bacteria may be quite inapplicable to others. According to Ehrlich, the specificity of antibodies is a result of differences in chemical structure of the antigens in response to which they have been developed. This view is strikingly borne out by the work of Obermeyer and Pick, and of Landsteiner and his co-workers who have shown that specificity can be modified by chemical procedures. Thus they have distinguished *structural* from *species* specificity. Proteins altered by physical processes, such as boiling, give rise to antibodies which react only with boiled protein; the latter, however, must belong to the species employed for immunization, that is, the species specificity is preserved. On the other hand, Landsteiner and Jablons have found that rabbits injected with acetylated horse serum develop antibodies which fix complement in the presence of acetylated serum protein from various other animals (hen, rabbit) in addition to the horse, but do not absorb complement with normal or boiled horse serum or with diazo- or nitro-serum protein. Rabbits will also produce an antiserum to acetylated rabbit serum. Accordingly, the process of acetylation has deprived the serum of those characters which distinguished it as belonging to the species horse, and has conferred on it a form of specificity which depends on the presence of the acetyl group (structure specificity). In respect of specificity, it is significant that a clear distinction exists between an animal's tissues and its serum (Fleischmann and Davidsohn), since antibodies obtained by injecting various organs do not fix complement in the presence of serum from the same species.

An antiserum to the protein constituent of haemoglobin does not fix complement along with serum, intact red corpuscles, haemoglobin, or acid serum-albumin (Browning and Wilson).

The antigenic power of different proteins varies extraordinarily; thus serum and red blood corpuscles readily produce antisera, while tissue proteins are feeble antigens, so that it is difficult to obtain antibodies to them even after many injections (Salus, Joannovics). Most purified proteins are found to have become seriously

impaired as antigens (Wells). Again, certain bacterial products, such as the haemolysin produced by streptococci, would appear to be altogether incapable of giving rise to antisubstances (McLeod). The question as to what particular character in proteins confers on them antigenic properties is a very subtle one, and obviously is of fundamental importance, but no satisfactory solution has been offered. There is certainly no constant correlation between toxicity and antigenic action, and the term "ictus immunisatorius" conveys little meaning; except by analogy it is not possible to predict the potency of a given antigen to develop antibodies.

While the specificity of antisubstances developed by immunization is so striking and constant, it must be remembered that similar highly specific bodies occur in serum altogether apart from the reaction to the introduction of an antigen: thus a lytic antibody for ox blood is present frequently in the serum of normal guinea-pigs. Accordingly, the development of an antibody need not necessarily possess any teleological significance. This consideration serves at least to rob of its apparently illogical character the formation of "heterogenetic" antibodies (Friedberger), as when a rabbit injected with guinea-pig's kidney develops an immune body for sheep's red blood corpuscles (Forssman). The introduction of certain foreign proteins into the tissues causes a metabolic change one of the constant manifestations of which is the development of this particular immune body. The capacity to produce a positive Wassermann reaction is probably a somewhat similar heterogenetic phenomenon; thus a property which is common to the serum of many normal rabbits (Browning and Mackenzie), appears in human serum practically only as the result of syphilitic infection. Such a constant phenomenon possesses great diagnostic value, but is probably to be regarded merely as the indicator of pathological processes.

Certain antibody reactions presenting points of special interest will now be considered briefly.

In *antitoxin reactions* the important fact has been recently elicited that the combination remains permanently reversible provided that a dissociating factor is introduced such as acid (Morgenroth and Asher) or alkali (Sachs).

In *agglutination and precipitation* the occurrence of zones, which is so characteristic of colloid interactions, is strikingly shown. Dreyer and Jex-Blake have proved that the explanation of zones as due to "proagglutinoids" does not apply generally. (It was supposed that the agglutinin, as the result of heating, became converted into proagglutinin, which possessed in marked degree the power of combining with the bacteria, but which had lost the property of causing agglutination.) Thus a suitably heated serum may actually exhibit increased agglutinating power and yet produce a marked zone, whereas a serum much weakened by heat may cause no zone. Further, zones may be produced by heating the bacteria instead of the serum. The analysis of the mechanism of agglutination reactions is complicated by the fact that the visible end result, agglutination, represents not merely the effect of a specific combination of the antibody with the bacteria, but also requires for its occurrence the co-operation of a suitable medium. Bordet¹ showed that, in the absence of a salt (or sugar), as when the bacilli are suspended in distilled water, no agglutination occurs, even although the agglutinin is bound; accordingly the loss of agglutinin by absorption from a serum is the true criterion of reaction. In certain instances agglutination depends on the action of complement as well as of antibody (Muir and Browning), and Bordet and Gay have shown that thermostable constituents of ox-serum (conglutinin) play a peculiar part in causing agglutination in the presence of antibody and complement.

Complement Fixation and Cytolysis.—Certain antibodies, when associated with the corresponding antigens, deprive fresh serum of its complement. The fixation of complement has been explained as due to adsorption. The mere occurrence of a serum precipitate is not sufficient to fix complement; thus, globin when mixed with any serum gives rise to a precipitate, but it is only when an anti-globin immune serum is employed that complement is bound (Browning and Wilson). Dean has shown that the amount of complement absorbed by a serum anti-serum complex may depend to a marked extent on

physical conditions, and varies according as the components are mixed simultaneously or the complement is added after the mixture of antigen with antibody has been allowed to stand for some time. It is, further, a noteworthy fact that with certain antigens, for example, serum, zones occur, so that if an excess of antigen is present complement fixation fails to take place; this is obviously of great practical importance. Complement fixation may occur without obvious formation of precipitate; and Arrhenius cautions against the assumption that adsorption takes place in such instances where there is not definitely a solid phase present.*

It is to be noted that the occurrence of adsorption phenomena may depend intimately on the chemical structure of the adsorbing agent, and a slight alteration in constitution may alter markedly the adsorptive property. For example, derivatives of cholesterol which fix lecithin, as tested by inhibition of snake venom lecithin haemolysis, do not differ from those which lack this power so far as can be measured by a physical character, such as the rapidity of precipitation out of emulsions with lecithin (Browning and Cruickshank).

Lytic Action.—Cytolysis may or may not follow complement fixation. A large amount of complement may be absorbed by the combination cell-receptor *plus* antibody, depending on the character and quantity of the immune body present as well as on properties of the complement peculiar to the species or the individual. For the production of lysis a certain minimum amount both of immune body and of complement is required, hence they do not behave as ferments; and there is also no satisfactory ground for regarding the using up of complement as a mere side phenomenon, methaemolysis (see Bordet³). As regards the part played by the immune body, Muir long ago observed that on increasing the amount of this component beyond the minimum quantity necessary to cause haemolysis, an increase was also in many instances produced in the amount of complement fixed by the immune body-receptor complex. It has been shown that this property of excess of immune body to lead to increased complement fixation depends to a great extent on the stage of immunization at which the antiserum is taken; thus, an immune body obtained from the rabbit after a single injection of washed ox blood has, when multiple doses are added to the corpuscles, only a feeble power of causing increased absorption of complement, whereas the "mature" immune body behaves as Muir has described (Browning and Wilson, confirmed by Thorsch). Thiele and Embleton describe further striking differences between mature and immature lysins. It is an important fact that the lytic power of a complement depends on the species of animal yielding the immune body through whose medium it is brought into combination with the red corpuscles (Muir); and this holds even when the immune bodies appear to combine with the same receptors of the red cells. The *deviability* of the complement of one individual may differ markedly from that of another member of the same species, and this property is quite independent of the lytic power. On what function differences in deviability depend has not yet been ascertained, but it is a point of the greatest practical importance in the interpretation of the results of complement-fixation experiments, and one which has not hitherto been sufficiently well recognized.† By procedures such as dialysis it is possible to reduce the deviability of complement greatly without effecting practically any alteration in its haemolytic power (Browning and Mackie). The complementing function of a serum is a complicated property and extremely labile, but it withstands the action of ammonium sulphate, and by making use of this reagent in addition to Liefmann's carbonic acid method for the purpose of fractionation much more uniform and satisfactory analyses have been obtained than by the carbon dioxide procedure by itself. Of course, it is not assumed that the various fractions so obtained are homogeneous protein entities, but the fact is to be emphasized that it is possible with great regularity to split complement up into four different components, and that certain combinations, consisting as a rule of at least three of these,

quantitatively reproduce the full haemolytic effect of the native serum (Browning and Mackie). This process of reconstitution is not a mere summation of effects, and the fractions do not correspond with the hitherto described end-piece, middle-piece, and third component

In general, the major portion of complement is represented by the following combinations of two constituents: (1) the pseudo-globulin components of each carbon dioxide fraction together, and (2) the pseudo-globulin of middle piece along with the serum-albumin. Frequently, however, the addition of the albumin to the mixture of globulins is required in order to reconstitute the complement, so that the co-operation of three components may be required. Thus, while the complement factors are distributed over the pseudo-globulins principally and may be wholly contained in these, the albumin also in many instances constitutes an essential element.‡

Attempts have been made to relate the inactivation of complement brought about by heat and other agencies to alterations in surface tension, but Schmidt¹ has shown that no inference can be drawn as to the complement action of a serum from its surface tension. In spite of much effort (v. Liebermann and v. Fennyvessy) no true substitute has been found for complement in the haemolytic system. On the other hand, it is a fact of great theoretical importance that immune body can be replaced by colloidal silicic acid (Landsteiner and Jagic).

It may be taken as definitely established that the component of serum which acts in this case is identical with the complement which causes lysis along with immune body (see Browning and Mackie); but the specificity which characterizes immune body is wanting, as silicic acid sensitizes various species of red blood corpuscles to the action of complement.

The alteration produced in the red corpuscles by immune body is no doubt analogous to that effected by silicic acid, but this does not elucidate the nature of the change, nor does the observation of v. Liebermann and v. Fennyvessy that corpuscles sensitized by immune serum are rendered more susceptible to the lytic action of soaps, explain the process of serum haemolysis. Certain lipoids—"lecithin"—may markedly modify complement action (Cruickshank and Mackie), but there is no evidence that lipoids can replace either immune body or complement in the haemolytic system.§ The difficulty which may attach to the analysis of haemolytic processes is well shown by haemolysis due to brilliant green along with serum (Browning and Mackie).

Ox blood treated with brilliant green is lysed by minute amounts of serum (0.005 c.cm. serum for 1 c.cm. 5 per cent. washed blood suspension) belonging to various species (human, guinea-pig, ox, etc.); but this result does not depend on complement, as Mackie has shown that serum diluted with ten volumes of physiological NaCl solution and then boiled still acts. The following is a summary of unpublished observations of Mackie on these points: The optimum effect is produced when the dye is allowed to act on the red corpuscles, and the latter are then well washed by centrifugalizing with 0.85 per cent. NaCl solution. Lecithin also lyses red corpuscles sensitized in this way. But the addition of small amounts of the dye to the lecithin emulsion or to serum deprives these agents of their "activating" effect. Egg-white does not lyse corpuscles treated with brilliant green; but serum precipitated with alcohol and repeatedly treated with alcohol and ether still acts. The amount of brilliant green which causes the sensitizing action is a minute fraction of the quantity which by itself causes lysis.

At present it is not possible to give any explanation of the phenomenon, but it affords a striking example of the subtlety of haemolytic reactions.

The Wassermann syphilis reaction probably belongs to the group of heterogenetic phenomena. No other non-specific alteration of serum, in the etiological sense of the term "specific," has been found to be so constantly present in any disease or so completely restricted to one particular condition. But the nature of the interaction between the constituents of a positive serum and the lipid "antigen" remains quite obscure in spite of many ingenious efforts at explanation. Our only means of

* Bechhold's observation that colloidal arsenic trisulphide ceases to be filtrable when mixed with a colloidal solution of Berlin blue, although the mixture is quite transparent, suggests, however, that colloidal solutions may act as adsorbing agents.

† Browning and Mackie have drawn attention to the value of the well-known method of freezing as a means of preserving a standard complement with constant deviability and lytic action over a considerable period.

‡ Euglobulin *plus* albumin often exert marked complementing action, but the addition of one of the pseudo-globulin fractions is required to restore the full power.

§ Chick has found (*Journ. of Path. and Bact.*, vol. xix, 1914, p. 130) that when pseudo-globulin of antidipteria serum is dialysed for a long time denaturation occurs, resulting in a precipitate of euglobulin, which ceases to form when most of the phosphorus has been removed from the soluble components, the latter retaining all the antitoxin. This observation is of great interest as regards the rôle of lipoids in relation to antibodies.

estimating it is through the occurrence of inhibition of that equally complex function complement.

The essence of the syphilitic "antigen," whether it be an emulsion of so-called lecithin or of a mixture of lecithin with cholesterol, is that in the presence of syphilitic serum it leads to the fixation of more complement than is fixed under similar conditions in the presence of normal serum; to speak of a pure syphilitic antigen conveys no precise meaning at present, since no chemical entity of the nature of "lecithin" has so far been prepared with certainty. Cholesterol, when added to the "lecithin" antigen, has the remarkable property of increasing the amount of complement fixed in the Wassermann reaction, as was first shown by Browning, Cruickshank, and Mackenzie; under suitable conditions this effect is strictly specific, and cholesterol causes no increase in the anticomplement action of the antigen alone or together with a non-syphilitic serum.

Wassermann and Lange have attempted to obtain an insight into the changes which lead to a positive reaction.

A positively reacting cerebro-spinal fluid rich in lymphocytes was centrifugalized so as to concentrate the cells; a small specimen of the cell-free fluid, and a similar specimen to which the concentrated cell content was added, were then heated for a prolonged period at 57° C. It was found that, as a result of this procedure, the specimen containing the cells reacted more markedly positive than the cell-free portion. Negatively reacting fluids containing cells, from cases of meningitis, were not rendered positive by a similar procedure.

The conclusion is that the reacting bodies may reside within the lymphocytes in syphilis. These suggestive experiments are well worth further analysis.

The question whether the various antibody reactions which may result from immunization with a given substance are really due to separate antibodies is important; the evidence goes to prove that a considerable degree of independence exists. Thus Muir and Martin have shown, in the case of bactericidal, opsonic and agglutinating properties of immune serums, that these functions vary independently; further, Mackie's observations on agglutination and complement deviation in the *coli* group point in the direction of independence of the antistances, as do also Hektoen's experiments in which dogs injected intravenously with rats' red corpuscles developed agglutinin and immune opsonin in their blood serum, while the cerebro-spinal fluid contained immune opsonin only.

The analysis of immunity reactions by mathematical methods (Arrhenius, McKendrick) has led to results which, if they could be accepted as general laws, would effect a most acceptable simplification. From the biological aspect, however, such generalizations cannot be regarded as universally applicable in the meantime. There are several reasons for this: (1) The active substances which are being measured—for example, in serum—are modified by the presence of a large quantity of matter which is prone to exert more or less unspecific effects.

Thus the action of complement is retarded or inhibited altogether when it is mixed with a specimen of the same serum which has been heated (Muir and Browning), yet it is certain the complement is not destroyed in the mixture, but merely masked—in this instance complementoid was shown to be largely responsible for the inhibition.

(2) Individual variations constitute an exceedingly well marked feature of immunity reactions.

The independence of deviability and haemolytic power of complement in the serum of different individuals of the same species illustrates this point well. Another striking example is the difference in affinity of antibodies for the respective antigens. Thus the immune body obtained from rabbits injected with ox blood has a high degree of affinity for the red cells, so that the treatment of the antiserum with the blood corpuscles removes multiple doses of the immune body; on the other hand, the normal antibody for guinea-pig's blood contained in ox serum has by itself little or no affinity for the red cells.

These variations among bodies closely similar in biological action, as well as the multitude of interfering conditions, seem to present an almost insuperable obstacle to the elaboration of general formulae.

Antagonistic Ferments (Abderhalden's Reaction).—Abderhalden's conception of antagonistic ferments proceeds from the fact that foodstuffs, especially protein, undergo a marked degree of splitting up in the intestine before they are absorbed into the tissues and there synthesized so as to become genuine constituents of the body. The reason for this process is supposed to be that the protein taken as food is foreign—that is, does not contain the proper proportion or association of the constituents required for building up the animal's own tissues; accordingly the foreign protein is broken down to such an extent

as will enable the intestinal cells to pick and choose the proper components in suitable amounts. On this basis Abderhalden has concluded that when foreign protein is introduced into the tissues parenterally—that is, otherwise than by the alimentary canal—the result is the development in the body fluids of ferments which break down the protein in a fashion similar to that which occurs in the process of digestion in the alimentary canal; in addition, the antagonistic ferment thus produced is supposed to be specific for the substrate in response to which it has been developed. Thus, in pregnancy the fetal elements of the placenta obtain access to the maternal circulation, and in virtue of their hypothetical foreign character they are supposed to lead to the production of ferments which digest placental tissue. For the detection of the products of ferment action two methods have been employed—(1) the altered rotation of the plane of polarization, resulting when the serum acts on a solution of peptone derived from the tissue in question, and (2) the demonstration of diffusible products—amino-acids—which pass into the external fluid when the serum and boiled substrate are allowed to act on each other in a dialyser; for this purpose Ruhemann's reagent triketohydrindenhydrate, "ninhydrin," has been mainly employed.

The differences in rotation observed by the optical method are generally small. With regard to the ninhydrin reaction, unfortunately a sharp line of demarcation cannot be drawn between the violet colour which is interpreted as a positive result and a complete absence of colour; an intermediate series of yellowish and reddish tints occurs, the true value of which it is extremely difficult to assess. Also, the dialysers vary considerably in their permeability for the substances under consideration.*

The cause of the Abderhalden phenomenon is not yet clear, and the gaps in knowledge regarding it are wide.

An attempt made† to correlate the phenomena with other immunity reactions, has led to the following results:

1. The reaction is a quantitative one; serum along with various proteins will produce diffusible substances reacting to ninhydrin, provided that sufficient serum is employed—for example, normal rabbit's serum acting on egg-white; (2) the reaction is diminished or abolished if the serum is heated sufficiently beforehand (60° C. for an hour); (3) the injection of an immunizing dose of egg-white (5 c.cm. 20 per cent. solution) intravenously into rabbits causes little or no alteration in the power of their serum to give a positively reacting dialysate when tested with egg-white seven to ten days later; (4) the lysis of washed ox blood by the fresh serum of an immunized rabbit gives rise to only a slight degree of proteolysis, as evidenced by the ninhydrin reaction when the mixture is dialyzed in the usual fashion; fresh normal rabbit's serum also gives rise to diffusible ninhydrin reacting substances when placed in contact with ox's blood corpuscles, although practically no haemolysis occurs in this case; heated immune rabbit's serum (one hour at 55° C.) along with ox's corpuscles did not yield a positively reacting dialysate. Normal guinea-pig's fresh serum, although it contains much more complement than rabbit's serum, does not necessarily cause a positive ninhydrin reaction when it produces lysis of ox blood sensitized with multiple doses of immune body from the rabbit.

Accordingly, there would not appear to be any constant correlation between the Abderhalden reaction and other antibody reactions.

As the results of many observers indicate, the diffusible bodies which react with ninhydrin are produced more readily in the presence of coagulated placenta by the serum in pregnancy than by the serums of normal individuals; but this is not an invariable rule, and also certain specimens of placental tissue yield a positive ninhydrin reaction with various non-pregnant serums. De Waele has come to the conclusion that the bodies which react with ninhydrin are derived from the serum and not from the substrate, while Plaut and Leitch independently have shown that serum on incubation with inorganic substances, such as kaolin or glass wool, without any organic substrate, may give rise to positively reacting dialysates; further, a given serum may develop the reaction in the presence of one of these inorganic bodies, and not with another. In view of these results, the specific nature of the reaction for antagonistic proteolytic ferments must be regarded as still extremely doubtful. The idea that in disease the body might produce antagonistic ferments to certain of its own organs opened out a

* This applies even to those dialysers which are supplied as standardized by the accredited dealers.

† Unpublished observations of Dr. McVey, carried out in the Pathological Department, Glasgow University.

wide prospect to investigators, and numerous remarkable results have been recorded; but granting even that the reaction possesses a certain degree of constancy as a phenomenon of pregnancy, the above mentioned facts render it highly inadvisable to conclude that a particular organ is affected solely on the ground that the patient's serum, along with that tissue as substrate, gives a positive Abderhalden reaction.

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ON THE THERAPEUTICS OF ORGANIC EXTRACTS.*

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It is perhaps curious that a physiologist should be asked to open a discussion on the therapeutics of organic extracts. But I think that it is not inappropriate, since the therapeutic value of every preparation must be tested by physiological methods. The normal body uncomplicated by disease is used, and it is further possible to isolate particular parts of the body and to note directly the effects, if any, which are produced. Again, the therapist must always start his investigations with the hope or expectation that a favourable action will be produced, while the physiologist is uninfluenced by this element of expectancy which is so fatal to impartial and unprejudiced observation.

Undoubtedly the observations of the therapist have frequently suggested the lines which experimental work must follow. No better example of this can be given than the discovery of the function of the medulla suprarenalis. Schäfer has described how Dr. Oliver found that

the administration of extracts of the suprarenal to his son produced sickness and constriction of the radial artery, and how he came to Schäfer asking that he should test the influence of the extract upon the blood pressure of an animal, with the results that when this was done its vaso-constrictor action was at once clearly demonstrated. Upon this observation followed the whole series of physiological experiments which have rendered our knowledge of the functions of the medulla suprarenalis so precise.

Probably the first hint of the production of what are now called internal secretions was given as far back as 1775 by Theophile de Borden. But it was Berthold who, in 1849, gave the first clear demonstration of their existence by his experiment of transplanting the testis into capons, and showing that such a transplantation induced the development of the characters of the sexually functional male.

It was not, however, till 1889, when Brown-Séquard presented to the Société de Biologie of Paris his observations upon the effects of the administration of extracts of the testes, that the attention of physiologists was directed towards the action of these internal secretions.

The work done before this date upon the thyroid should have suggested the possibility that such products are formed. Gull, in 1873, had described the condition of myxoedema; and in 1879 Ord had definitely associated it with changes in the thyroid. Kocher and the Reverdins, in 1882, had described a similar condition following removal of the thyroid, and Schiff, in 1884, had demonstrated that the thyroid might be transplanted in the dog, and that the onset of the symptoms of removal of the gland might thus be prevented. The work of Vessale and Gley in 1891, showing that the intravenous injection of extracts of the gland act in the same way, and that of Murray proving that glycerine extracts administered by the mouth are effective, were the natural sequence of these observations of Schiff. The old theory, maintained to this day in a modified form by von Cyon, that the thyroid regulates the supply of blood to the brain, had to be abandoned when it was shown that the mere administration of extracts could prevent the onset of untoward symptoms after the gland was removed.

Apart from the thyroid—with which we are not at present further concerned—Oliver and Schäfer in 1894 in this country, and somewhat later Cybulski and Szymonowicz described definite symptoms, the result of the injection of extracts of the suprarenal bodies.

Since then an enormous amount of work has been done upon the action of these extracts, and one important result has been to explode some of the older views as to the part played by these ductless glands and to throw important light upon the real nature of their functions. For instance, the idea that such structures as the suprarenal act by destroying a hypothetical poison had to be abandoned in the light of the more recent knowledge of the mode of action of extracts of these bodies. It is the study of these extracts which has clearly demonstrated that the various ductless glands—to use a not very satisfactory generic title—have for their function the preparation of internal secretions which each produces a specific effect upon the body, either increasing or decreasing the activity of various organs.

Naturally, their exciting or augmenting action was at first the more manifest, and this led Starling to name them "hormones." But since some at least depress activity, the name is unsuitable. Schäfer and others have tried to devise other names indicating the particular mode of action, but personally I fail to see the advantages of such clumsy Greek terms, when the convenient generic title, "internal secretion," is available.

But while the study of these extracts has so markedly advanced physiological knowledge, it must be recognized that this study is fraught with danger, and that it has led many investigators astray, and has produced a very large crop of errors. The dangers are of several kinds.

1. Many extracts of organs are little better than dirty mixtures of the organic and inorganic constituents of the organ in a more or less decomposed condition, and their administration is a return to the dark days of polypharmacy. Because they purge or influence the blood pressure, there is no reason to conclude that their action is due to a specific product of the organ—to a particular internal secretion.

* Discussion in the Section of Pharmacology, Therapeutics, and Dietetics of the Annual Meeting of the British Medical Association at Aberdeen, 1914.