THE CAPILLARY ENDOTHELIUM IN RELATION TO ANTIBODIES.

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INTRODUCTION.

Importance of Endothelium.

PATHOLOGISTS and physiologists are agreed that the capillary endothelium is of high importance in the animal economy, though it is admitted that its functions are involved in much obscurity. With the immunologist, the primary difficulty is lack of physiological data which would enable him to start with the normal functions and to interpret abnormalities in the light of these. And it is obviously far from easy to plan experiments providing the sort of information the immunologist wants about the functions of endothelium as part of a living mechanism, in which parenchymatous cells, body fluids, and endothelial channels jointly participate.

It would, however, be an exaggeration to say that the functions of the capillary filter in relation to immunity are completely unknown.

In the first place, histologists have devoted much attention to the characters of endothelium in inflammation and in chronic bacterial infections. When histological preparations show visible changes in the morphology and permeability of an endothelial lining, it is safe to assume that these cells are also being

https://doi.org/10.1017/S0022172400008305 Published online by Cambridge University Press

subjected to finer and invisible modifications, of a chemical and physical nature, which alter their relations to the material which comes into contact with them and to the fluids passing through them.

For example, an endothelial lining forms a barrier which bacteria must penetrate before they can make their way either from the tissues into the circulation or in the reverse direction. Infection and resistance often depend on the impairment or integrity of this barrier. At the focus of infection in a susceptible animal there is usually an inflammatory reaction, which is accompanied by visible alteration in the endothelium and is due to toxic products of the bacteria and perhaps also to the irritant action of disintegrated tissue. A further influence may be operative if the local lesion is bathed in a serous exudate. It is known that such exudates may be rendered toxic by modifications attributable to the action of adsorptive agents. Hence the bacteria, if present in sufficient numbers to adsorb effectively, may transform the exudate into a toxic principle which irritates the endothelial barrier, makes it permeable for the bacteria, and so allows them to gain access to the circulation. This action by adsorption may sometimes help to explain the acquirement of invasive and parasitic powers by bacteria which more usually live as saprophytes on the surface of mucous membranes.

On the other hand, inflammatory change in an endothelial barrier may affect its permeability in such a way that antibacterial fluids, which would be held back by normal endothelium, are allowed to pass into the infected focus and thus assist recovery. This may also help to explain "non-specific immunity"; *i.e.* a foreign protein which is quite unrelated to the protein of the invading bacteria may, by irritating the endothelium, promote the passage of antibacterial substances, which appear promptly, before ordinary antibodies have had time to develop.

Another example may be taken from tuberculosis. Histologically, the relations of endothelium to tubercle bacilli have been the subject of many observations, including those of the Royal Commission on Tuberculosis[1] and of Foot[2] who traced these cells by the ingenious method of injecting intravenously colloidal suspensions of carbon. Apart from association of endothelium with the proliferative and degenerative tissue changes in tubercle formation, the histologist finds conspicuous differences in the reaction of mammalian and avian endothelium to mammalian and avian tubercle bacilli, and, amongst different species of mammals, differences in the behaviour of endothelium towards the same type of mammalian tubercle bacilli. All this suggests that differences in susceptibility are associated with certain differences in permeability and resistance on the part of endothelium, which have not yet been explained in terms of chemical and physical properties.

In natural immunity against a particular bacterium, *e.g.* the immunity

[1] *Royal Commission on Tuberculosis* (*Human and Bovine*). Vol. v. of Appendix to Final Report, pp. 276–83 and 298–300. Cd. 5975. 1911.

[2] *Journ. Exper. Med.* xxxii. p. 513 and p. 533; xxxiii. p. 271. 1920 and 1921.

of the fowl towards the anthrax bacillus, it is known that antibacterial substances of some sort or other must be present in the body fluids; but they cannot be identified with ordinary antibodies and are not demonstrable in serum obtained by bleeding the animal. The probability is that these substances are extremely labile and are constantly being produced, inactivated or broken up, and formed again in the course of the animal's ordinary metabolism. How is the nature of these substances affected by passage through the endothelial filter of the animal's capillaries? In other words, is the difference between a naturally immune animal, such as the fowl, and a susceptible animal, such as the rabbit, due entirely to primary differences in the body fluids, the endothelial filters being merely passive, or is it partly due to the special selective activity of the endothelium? No categorical answer can be given, because the endothelium and the body fluids are parts of one and the same mechanism, and it is impossible to decide by experiment what functions are attributable to the former alone; but it seems likely, from analogy with filtration by other types of living cells, that the properties of the endothelial filter may differ in the two animals. One may note here the marked difference between the living plasma and the serum, which is removed from the influence of the capillary filter. The fowl's serum is not bactericidal towards the anthrax bacillus, but the rabbit's is, though the former is the immune and the latter the susceptible species.

In addition to the general question of the selective permeability of endothelium in the normal, the infected and the immunised animal of a particular species, there is evidence, from the phenomena of local immunity and susceptibility, that there are local differences in the endothelium of the same animal.

There is another line of investigation which is of much interest. Recent work on anaphylaxis has raised a strong presumption that the endothelium is the site of that union between anaphylactic antibody and antigen which produces shock. Hence one is led to consider whether these cells may not also be concerned with other immunological processes which are not necessarily attended by shock.

The above examples will suffice to indicate that the relations of capillary endothelium to immunity constitute a problem which has definitely come within the sphere of practical interests. One cannot afford to dismiss it on the ground that it is one of the many mysteries of the animal body which appear, at present, to be insoluble, and that, therefore, it would be economy of effort to divert attention to more easily explored channels.

Antigen and antibody are generally used as complementary terms, implying that it is impossible to study them separately; but it is possible to focus more attention on the one than on the other, and I think it is characteristic of most recent work on theories of immunity that interest is focussed on the antigen, the antibody being utilised mainly as a mirror to reflect antigenic properties. I refer to such questions as the serological subdivision of bacterial species.

What is aimed at is a classification of antigens; the antibodies are, to a large extent, subsidiary to this purpose, in relation to which questions do not arise as to what particular cells (endothelial or other) participate in antibody formation; and, therapeutically, the problem is simply to find the right antibody for the right antigen. Closely associated with this work is the attempt to correlate antigenic structure of bacteria with their virulence. The satisfactory feature of work on these lines is that it is based on laboratory experiments which can generally be planned so as to give unequivocal conclusions, irrespective of problems about the mechanism of antibody formation.

Important as this work is, it needs to be supplemented by the adoption of a different outlook, in which the antibody is of main interest and the antigen is merely a means of stimulating the production of the former or of identifying it. To say that the animal body resists bacterial invasion by the formation of antibodies is a postulate which needs explanation. It is often impossible to demonstrate antibodies in a naturally immune animal; in susceptible animals, successful resistance is not always associated with the production of any known antibodies, and, when it is, it is often impossible to prove that these antibodies are the humoral elements in the mechanism of recovery.

How are antibodies formed and what are their attributes? Once formed, their existence, whilst it continues, is independent of the antigen which stimulated their production, and they must have many characters not comprised within the statement that they were produced by, or will react with, a certain antigen.

These considerations lead to others. What is the real basis of the distinction between "specific" and "non-specific" antibodies? Further, is it correct to assume that the humoral element in resistance to bacterial invasion is always an affair of antibodies, in the current immunological sense of the word? There are also difficulties about the well-known antibodies, such as antitoxins, precipitins and bacteriotropins. They are not always formed as a matter of course and in the anticipated quantity, when a suitable animal is treated with the usual dosage of the "right" antigen; and, when their output is charted, the antibody curve presents curious oscillations, for certain of which no satisfactory explanation has yet been found. All these questions imply that there are serious gaps in what is at present known about antibodies, and that an endeavour must be made to fill up some of them.

In attempting to explore this difficult territory one has to deal with physiological factors, the exact nature of which is largely hypothetical, since there are not sufficient data to translate them into the more concrete terms of chemistry and physics. One must start with some hypothesis, ascertain if it is compatible with known facts, and, if it seems to be, consider whether it helps to explain them.

What cells of the body are most likely to be the site of antibody production? This is an old question; many attempts have been made to associate this function with some particular organ or organs, *e.g.* spleen, liver, lymphatic

glands, etc., but it cannot be said that they have succeeded. As it is known that antibodies may be produced locally in many different sites of the body, it does not seem very likely that their manufacture is the function of any central organ. But endothelium is ubiquitous. That is one reason why it has a particular claim for consideration. This claim does not necessarily imply that endothelium may secrete antibodies in just the same way that special organs secrete special enzymes; antibodies may be the product of a complex interaction between cells (perhaps of more than one type) and tissue fluids. All that need be implied in this hypothesis is that endothelium takes an important, not necessarily an exclusive, part in the formation of antibodies.

Method of Enquiry.

In searching for avenues of approach to an obscure subject, it is useful to start with some idea of what is likely to be helpful.

Filtration is a normal function of endothelium; so it seems to me that, if endothelium participates in the production of immune bodies, its mechanism will probably have something to do with filtration, filtration modified by the adsorption of foreign protein. Do immunological data lend any support to this hypothesis? Preliminary consideration leads me to think that they may do, provided that one can formulate a conception of immune bodies which is compatible with this method of their production.

The idea that antibodies are simply fixed and immutable counterparts of antigens is not sufficiently elastic. Some additional hypothesis is required. I think the simplest is the idea that antibodies in the living body of the actively immunised animal are not necessarily identical, as regards activity and combining properties, with the antibodies demonstrable in that animal's serum, and that the differences, when they exist, are attributable to the transition from the unstable and more complex conditions of the living plasma to the relatively inert and stable conditions of the serum.

These two ideas I have utilised as clues in pursuit of the enquiry.

Proceeding to examine certain data derived from observations on anaphylaxis, the production of antitoxins, and the phenomena of local immunity, I think one can find suggestions which justify a further effort to elaborate the view that the capillary endothelium participates in the production of antibodies.

After developing this hypothesis, I call attention to its limitations in relation to any comprehensive theory of immunity.

EVIDENCE FROM EXPERIMENTAL ANAPHYLAXIS.

Laboratory Data.

These experiments may be described in general terms as studies *in vivo* of the precipitin type of antibody reactions. From the very large mass of work on this subject I propose to select certain data which appear relevant to the present discussion on the production and nature of antibodies.

From this point of view, the typical experiment in passive anaphylaxis consists in the injection of a foreign protein (A) which is "ear-marked"; it contains a precipitin and hence its relation to the animal's tissues can be detected by subsequent introduction of the corresponding precipitinogen (B), when reaction between the two produces shock. From such experiments it can be shown that A has entered into certain relations with the tissues before B is introduced and it is natural to assume that, as foreign protein, it would have entered into these same relations in the absence of this particular "ear-mark." Thus passive anaphylaxis provides useful information, quite irrespective of the events leading to anaphylaxis, as to the fate of foreign protein when introduced into the animal body parenterally, *i.e.* it reveals something about the behaviour of antigens.

Similarly, active anaphylaxis shows, by means of a shock dose of antigen, that in the process of immunisation antibodies are stored in certain situations. This storage precedes the shock and would occur in just the same way if the conditions necessary for anaphylaxis were not fulfilled. Hence the information about antibodies which is thus acquired is of general utility, the anaphylactic experiment being merely the instrument for its discovery.

These are the two main points which I have in mind in the following references to work on anaphylaxis. My material is derived from Doerr's recent survey of the subject[1].

Many of the long and tedious controversies have now been settled. It is no longer necessary to weigh rival theories as to the humoral or cellular location of anaphylactic shock, nor has any interest survived in the explanation furnished by a mysterious substance called "anaphylatoxin." It is admitted that anaphylactic shock is essentially a cellular phenomenon and that the anaphylactic antibody is a precipitin.

Doerr sees reasons for thinking that the particular cells in which the shock antigen-antibody reaction takes place are not the parenchymatous tissue but the capillary endothelium. Before quoting his arguments, it is appropriate to note that the same suggestion was made many years ago (1910) by W. M. Scott. In his studies of anaphylaxis in the rabbit[2], he examined the various possible ways in which shock might be produced and arrived at the following conclusions: "By a process of exclusion we are forced to postulate an injury to the capillary walls themselves for the production of the anaphylactic effects. Objective evidence of this is not easy to furnish. Histological methods of greater delicacy than have hitherto been applied are required to bring to the eye such changes in the cells concerned—which cannot, after all, considering the rapidity of recovery, be of a gross character. The prevalence of capillary haemorrhages is of course suggestive."

To return to Doerr, he states that, so far as he is aware, there are no

[1] Weichardt's *Ergebnisse der Hygiene, Bakteriologie, Immunitätsforschung und experimentellen Therapie*, vol. v. pp. 71–274. 1922.

[2] *Journ. of Path. and Bact.* xv. p. 31. 1910.

https://doi.org/10.1017/S0022172400008305 Published online by Cambridge University Press

facts which are incompatible with this idea of the part played by the endothelial cell, but there are many observations which are in accordance with it. For example, he quotes the experiments of Dale who found that the isolated normal uterus could not be passively sensitised by merely placing it in a bath containing antibody but it was necessary to perfuse the vessels of the organ for 5 hours with dilute antiserum. "Further there are the frequently repeated experiments which show that the organ cells of sensitised guinea-pigs are unable to fix the corresponding antigen *in vitro*. Again, there are the changes of the blood in shock, the predominance of vascular functional disturbances amongst the symptoms of anaphylaxis, etc."

He quotes some interesting observations on the time required for sensitisation in passive anaphylaxis. Guinea-pigs were sensitised intravenously with the minimal dose of antiserum and during the first stages of the latent period about half their total volume of blood was removed and replaced by normal blood. If the withdrawal of blood took place within the first hour, the anaphylactic condition was not produced; removal after the lapse of one hour or at a later period had no effect; the animal became as markedly hypersusceptible as the control which had not been bled. Owing to the shortness of time required for the union of cells with the serum containing anaphylactic antibody, it appears to Doerr improbable that the antiserum passed through the vessels and entered the parenchymatous tissue; "one would rather think of the capillary endothelium as the site of union, of activation, and of the antigen-antibody reaction."

It has been estimated that the amount of antibody taken up by the cells during the first hour is about 40 per cent. of the quantity injected; the surplus remaining in the blood apparently plays no further part in the sensitising process. After this first union, there is the well-known latent period (24 hours or less) before the anaphylactic condition is established. During this time "activation" of the cellular antibody is brought about. Doerr remarks that this second phase does not mean that time is required for the gradual accumulation of antibody up to the requisite concentration, since Fenyvessy and Freund had shown that the latent period was not abbreviated if 3–4 multiples of the minimal dose of antiserum were injected; with the larger dose, it was proved by titration that about 40 per cent. of the total was fixed by the tissues in one hour, *i.e.* a much larger quantity than after the minimal dose. According to Doerr, the "activation," for which a latent period is required, does not imply that a qualitative change has been produced in the antibody but that there has been a slow transition (as is commonly the case in colloidal reactions) from loose to firm union between antibody and the shock cells.

The sensitising antiserum is a foreign protein and its antibody component does not split off from the rest of the protein when the serum is taken up by the cells; both disappear from the circulation simultaneously. But Doerr does not think it probable that the foreign protein passes through the cell membrane and permeates the cell protoplasm, because, if it did, it would

damage the cell and be incompatible with the latter's continued vitality; but passive sensitisation does not lead to extensive tissue necrosis; in fact hypersensitiveness depends on the maintenance of the vitality and reactive capacities of the sensitised cells. Hence he concludes that what actually takes place is attachment of the antiserum to the surface of the cell by adsorption, "activation" being a secondary fixing of this adsorbed material.

In spite of careful experiments by Doerr and others, it had been found impossible to obtain "reversed anaphylaxis," *i.e.* the passive anaphylactic shock cannot be produced if the antigen is injected first and the antibody afterwards. What is the reason? Is the antigen modified by the cells in such a way that it becomes incapable of subsequent reaction with antibody? Or is it because the antibody has to participate in some change before its union with antigen can provide the stimulus causing anaphylactic shock? Doerr adopts the latter view, the postulated change being the "activation" referred to above. "Only the antibody enters into that intimate relation to the shock cells which is necessary for the reaction, not the antigen."

Experiments on "antisensitising" are of interest. It has been shown that guinea-pigs can no longer be passively sensitised with rabbit immune serum if they have previously received subcutaneous injections of normal rabbit, sheep, dog or human serum. After small doses of serum, the protective action is produced slowly (after 8 days); large doses (8 c.c. spread over 4 days) produce resistance more quickly (in 1–3 days after the last injection). When the refractory condition is once established, it persists for a considerable time (at least for 68 days). What is the explanation of this phenomenon? As the reaction is non-specific, one can hardly postulate the formation of "anti-antibodies." Doerr is inclined to the view that the guinea-pig's cells were "saturated" with the first doses of foreign protein, so that the subsequent immune serum could no longer enter into relation with them. In support of this view he quotes experiments of Weil's, who showed that, though guinea-pigs treated with large doses of rabbit, sheep, or human serum resisted heterologous passive sensitisation, they could be passively sensitised without any difficulty when homologous antiserum was used (the serum of hypersensitive guinea-pigs). Apparently the animal's cells had no difficulty in taking up the antibody contained in the proteins of their own species.

Doerr discusses the view that, on the cellular theory of anaphylactic shock, circulating antibody must be protective, by neutralising antigen on its introduction and before it can unite with antibody localised in the cells. In support of this, Weil showed that actively or passively immunised guinea-pigs could be made insusceptible to the introduction of antigen if a good dose of rabbit antiserum were introduced intravenously shortly before the antigen. These results were confirmed by others, but it was found that their significance was open to question. Friedberger and Hjelt showed that, if guinea-pigs were sensitised with anti-horse or anti-cat serum obtained from rabbits, the anaphylactic condition could be temporarily put in abeyance by inoculating

1 c.c. of normal rabbit serum 24 hours after the passive sensitisation. Doerr quotes a similar instance from active anaphylaxis, where this effect was produced by rabbit serum but not by the serum of other species. The effect persisted for at least 15 hours in active anaphylaxis and for at least 24 hours in passive anaphylaxis; it was demonstrable very soon after the introduction of the antagonistic normal rabbit serum. In view of such non-specific effects, one has to be cautious about postulating a protective action of circulating antibody. "In the much greater proportion of cases, free and cellular antibody co-exist in the organism, both in active and in passive anaphylaxis; only the relative quantities of the two vary within wide limits." Moreover, "the neutralisation of precipitinogen by antibody is not completed either suddenly or without leaving a trace behind. Even if antibody is present in considerable excess, free antigen remains in the mixture and may enter into reaction with freshly added antibody or with antibody which is differently located."

The condition of (1) passive susceptibility to anaphylaxis, when conferred by injection of heterologous antiserum, persists unaltered for some days; the hypersusceptibility then diminishes and finally disappears from the 6th to the 10th day. But, if (2) the antiserum is homologous, the condition lasts from 60 to 70 days. In (3) active anaphylaxis it generally remains for more than a year. (1) may be explained by the assumption that the foreign protein, together with its contained antibody, becomes inactive as soon as the organism has formed an antibody against this protein. Hence "a co-existence of antigen and antibody is possible *in vitro* and in the blood-stream but not in the cells, where one of the reacting components excludes the presence of the other." In (2) the protein is homologous, so there is no reason why it should be turned out of the cell so readily. But why the marked difference between (2) and (3)? Doerr admits that this is a puzzle and discusses it as follows: "Is there such a great difference between the protein of species and of individual? Or is the mode of union between cell and antibody introduced from without less firm than between cell and autochthonous antibody? And what is meant by autochthonous? Does it mean that the antibody only arises in the like organism or that it arises in the anaphylactically reacting cells? Site of production and localisation of antibody are not necessarily identical; even in actively sensitised animals, the antibody might, for example, be produced in the lymph nodes and then form a secondary attachment with the smooth muscle fibres, so that the reacting tissue would be only passively sensitised."

In concluding these quotations from Doerr's article, I note that he makes a suggestion which I have already raised at the commencement of this section. Passive anaphylaxis might be utilised to study the fate of foreign protein in the animal body. "Its suitability for this purpose is provided by the circumstance that antibody is united with protein and is broken up along with the latter and that the anaphylactic antibody is the only one which admits the ready demonstration of its presence in, or attached to, fixed tissue cells."

Comment.

In dealing with general questions of immunity, it is not quite clear whether anaphylaxis should be treated as a main problem or as a side issue. Until recently, the tendency has been in the former direction, and there has been much theoretical discussion about the relation of anaphylaxis to "Allergie," which, being an altered state of tissue reaction caused by the introduction of foreign protein, was supposed to be the preliminary stage of immunisation. Since then a reaction has set in. Theories about "Allergie" have tended to assume a merely academic interest. It is realised that a good deal of the controversial work about "anaphylatoxin" and the like has been wastage, and that one may rest content with the view that shock is due to a reaction between precipitin and precipitinogen which takes place in certain cells of the animal body. Hence the tendency to leave anaphylaxis alone, as being an interesting but not specially important phenomenon, the nature of which has been duly explained.

Whichever view be adopted, there is one curious feature which is worth considering. How is it that, unless obvious precautions be neglected, accidental anaphylaxis is a rare event? As Doerr has shown, it cannot be because there is usually a supply of circulating antibody which neutralises the antigen before it can reach the cells. Shock may occur in spite of the presence of such circulating antibody; on the other hand, shock may be absent under conditions where there is antibody in the tissues but not in the blood stream. Amongst the many factors upon which absence of anaphylaxis in active immunisation probably depends, is one of them attributable to a difference between the condition of antibody in the serum and its condition in the plasma and cells of the living body? If there is a difference between the two, the latter condition may not always be appropriate for a precipitinogen-precipitin reaction, and anaphylaxis may only occur when the cellular antibody is of the serological type.

Doerr, if I interpret him rightly, does not see any need for this hypothesis. He states that antigen and antibody may co-exist *in vitro* and in the circulation but not in union with cells. This may be true in some cases but it is rather puzzling in others. Admittedly, the work on anaphylaxis provides strong evidence that a precipitin and its antigen cannot co-exist in union with the same cell as independent and compatible entities, since anaphylactic shock would take place. But how are antigen and antibody kept apart in active immunisation, when shock does not occur? One must suppose that the antigen comes into close contact with the cell which it stimulates to form antibody. How is it possible, during the continuation of this process, to avoid contact between antigen and cellular antibody? It would seem arbitrary to postulate that special colloidal conditions prevent that sort of contact which would lead to interaction, and I think some other kind of explanation is needed. Just as certain physiological products, such as enzymes, may have an antecedent form

which is not active, so it is possible that the antibody, before being turned out of the cell, may be in a quiescent stage which precedes its conversion into a precipitin.

If there are these two phases in the constitution of an antibody, does the change from the first to the second take place in the circulating plasma or in the serum? If the former alternative is correct, there would appear no reason to doubt the ordinary serological tests for circulating antibody. But if the change does not (or may not) take place until the serum has been collected, there will obviously be a different explanation for the apparent co-existence of circulating antigen and antibody in the actively immunized animal; the antibody may not have assumed its precipitin type.

Some information about the behaviour of antigen can be obtained from experiments in passive anaphylaxis where a foreign protein, which must necessarily behave as an antigen, can be identified owing to its content of anaphylactic antibody. It appears, from this method of observation, that with some antigens adsorption by the cells takes place very rapidly (in about one hour), that a change, probably a firmer union with the cells, occurs during the first 12 hours or thereabouts (latent period in passive anaphylaxis), and that the adsorbed antigen is broken up and presumably ceases to function as antigen in from the 6th to the 10th day (termination of anaphylactic condition). As the cells are not damaged by these events, there is a strong probability that the foreign protein does not penetrate them but simply acts on their surface.

Can anaphylaxis provide any further information about the effect of this action upon the cell? The impossibility of obtaining "reversed anaphylaxis" shows that the first foreign protein modifies the cell in such a manner that the latter does not unite with the second protein (containing "anaphylactic antibody") in the same way, since it no longer makes with it that firm union which is the necessary prelude to shock. Again, the "antisensitising" experiments show that in the course of a few days (five or more) the first foreign protein impresses a definite change on the cells which have adsorbed it. They behave in a different, though non-specific, manner when the second protein is introduced, the evidence being, as before, that they do not make with the latter the firm union necessary for shock. The postulate that the cells are "saturated" with the first protein seems unsatisfactory. This explanation is not valid when the carrier of the precipitin is not foreign but homologous protein, since shock is obtainable under these conditions; and it is difficult to imagine how the first foreign protein could remain linked to the cells (still less "saturate" them) for so long a period as 68 days. Moreover, there is no evidence that the cells of an animal which has formed antibody in response to a particular antigen are "saturated," since the animal is quite capable of producing other and different antibodies when their corresponding antigens are subsequently injected.

I think the main conclusion is that cells which have adsorbed foreign protein are changed in some way which has not been clearly defined, and that

this change tends to persist after the disappearance of the foreign protein. This evidence, derived from anaphylaxis, of the acquirement of a new property by the cells is in accordance with the fact that cells which have ceased to turn out a particular antibody may be caused to renew their supply of it by the injection of a non-specific stimulus.

Why is the condition of anaphylaxis produced by active immunisation of much longer duration than the passive condition induced by injection of homologous antiserum? In the former case, the protein constituents within the cell which are called "anaphylactic antibody" are living material which is renewed from time to time in the course of the cell's metabolism; in the latter case, the protein which acts as antibody is dead and therefore incapable of renewal, though, being homologous and non-irritant, it resists disintegration for a much longer time than foreign protein would. If this is a satisfactory explanation, it may have a more general application to immunity. Is the long period of immunity which follows infection or vaccination with certain viruses due to the creation of living cellular antibodies which propagate themselves? And when susceptibility returns in a relatively short time after recovery, does it mean that the antibodies, though built out of the animal's own protein, are incapable of self-renewal as living material and therefore disappear in the normal processes of protoplasmic repair?

Doerr's reasons for thinking that the endothelial cell is probably the site of anaphylactic shock are of obvious interest in relation to the wider questions of the immunological functions of endothelium, provided that one does not place undue weight upon the data. The suggestions which they furnish do not amount to proof. As he points out, mere evidence that antibodies are closely associated with endothelium does not exclude the possibility that they were manufactured elsewhere and subsequently adsorbed by these cells. All that can be said definitely is that anaphylaxis provides information about the interactions between foreign protein and certain cells of the body and that this information does not appear incompatible with the hypothesis that endothelium participates in the formation of antibodies.

The anaphylactic test, as has been noted, provides an ingenious method of "ear-marking," and thereby identifying, a foreign protein which is used as an antigen. One naturally asks whether it is possible to find other methods of achieving the same object. I wonder if the biochemists will ever be able, by using an artificial antigen containing a known chemical substance with specific antigenic properties, *e.g.* an iodo-protein, to identify that substance by microchemical methods in histological preparations of the immunised animal's endothelium. If so, the microchemical reactions at different stages of immunisation might throw some light on the mechanism of antibody formation.

THE FORMATION OF ANTITOXIN.

Diphtheria Antitoxin.

It is of interest to consider whether the hypothesis now under discussion is compatible with what is known about the formation of antitoxin. I take diphtheria antitoxin, as being the best known and the most important. There is also the advantage that the data which are of special interest in this connection have been recently reviewed by Madsen in his first Harben Lecture for 1922[1] on specific and non-specific formation of antibodies.

Madsen raises several important questions about the reactions of a horse which already possesses some diphtheria antitoxin in its blood.

When a horse receives a further immunising dose of toxin, there is a "strongly pronounced negative phase of three days' duration, after which the antitoxin concentration rises until it reaches a maximum on the ninth day, and then falls again." On the ninth day the formation of antibodies has "practically ceased" and "the factors destructive to the antibodies can now have full play." The destructive factors, he states, have been present all the time but have been concealed by excess of production. "This phenomenon, shown by the active immunity curve, may be compared with what is observed if one throws a stone upwards into the air; the force of gravity, which is continually acting upon it, is counteracted by the upward driving force, but only up to a certain height, when the stone will again fall down."

Why is injection of toxin into such an animal followed by the appearance of the negative phase? It cannot be explained by the hypothesis that part of the antitoxin present is neutralised by the new dose of toxin. "A simple calculation will suffice to show that if we have to deal with a horse possessing, for instance, a concentration of about a few hundred antitoxin units in its blood, a few c.c. of this will suffice to neutralise even the maximum amount of highly potent diphtheria toxin which we are able to inject into the horse." Madsen points out further that the maximum decrease is not observed at once, as would be expected if the antitoxin were neutralised, but not until one or two days after injection. He suggests that the effect may be due to "some inhibitory action on the antitoxin-producing cells."

Again, why is this fall in antitoxin followed by a rise? This fact also is obviously incompatible with the hypothesis of neutralisation such as occurs *in vitro*. "If a few c.c. of blood are withdrawn from the horse and mixed with the toxin, and this again injected into the horse, no reaction occurs, either in respect to fever, local infiltration, etc., or to antitoxin formation." There is a corresponding difference between active and passive immunity. The actively immunised horse reacts strongly to a fresh subcutaneous injection of toxin "both in regard to general reaction and production of antitoxin," whereas the passively immunised animal, possessing only a slight concentration of antitoxin, shows no reaction to the same amount of toxin.

[1] *Journ. State Med.* XXXI. Feb. 1923.

https://doi.org/10.1017/S0022172400008305 Published online by Cambridge University Press

Madsen considers that the most satisfactory explanation of antitoxin formation "seems still to be the view advanced by Salomonsen and myself in 1896, namely, that *the cells of the organism under the action of the toxin may be supposed to acquire a new functional capacity, that of secreting antitoxin*, each new injection of toxin acting as an incitement to antitoxin production."

He then proceeds to point out that the injection of specific antigen is not the only means of obtaining an increased production of antibody. It may be brought about by a variety of non-specific stimuli, such as removal of existing antitoxin by repeated bleedings, the production of an inflammatory condition, or the action of various chemical substances. "On the basis of the conception of antitoxin formation as a sort of secretory process, Walbum thought it probable that it might be acted upon by substances possessing a catalytic action, such as, for instance, certain *metallic salts*, and by a closer study this proved to be the case." The remainder of Madsen's lecture is mainly occupied with records of experiments which illustrate this point.

In the first experiments on horses the endeavour was made to estimate the influence of one of these salts, manganese chloride "*after* the action of the toxin had ceased to prevail." When the antitoxin content of a horse had fallen to 160 units per c.c., small intravenous doses of the salt were given daily. "The antitoxin concentration showed a rise from 160 a.u. to 350 a.u. per c.c. as a consequence of the metallic salt alone, without toxin." In another example, the antitoxin content was raised from 700 to 1000 units.

Similar effects were observed when horses received continued treatment with both toxin and the metallic salt. A horse had persistently refused to yield a maximum of more than 200 units when treated with toxin alone; when daily injections of manganese chloride were commenced "simultaneously with immunisation with diphtheria toxin in the usual way," a content of 375 units was eventually obtained. This case was corroborated by others, which justified Madsen in making the general statement that "there is thus no doubt that by the application of metal salts, more especially manganese chloride, we are able to produce a rise in the antitoxin production."

Another point of great interest is the rapidity with which various metallic salts produce their effect. Referring to an example from his antitoxin curves, he says: "It will be seen that, following a single injection of magnesium chloride the antitoxin content of a horse immunised shows an enormous rise, reaching the maximum in the course of one hour. The rapidity of this rise is quite startling, and the relation is here quite different from that of the usual antitoxin curve appearing after toxin injection, since the negative phase is totally absent." Madsen considers that these experiments support the conception of antitoxin formation as being a secretory process, though he regards it as an open question whether it is possible to form new antibodies with such rapidity or whether the real explanation is that antibodies previously lodged in the organism are suddenly pushed out into the circulation under the action of the salt.

In addition to his observations on antitoxins, I may note that Madsen also calls attention to the stimulating effect of metallic salts on the production of other types of antibodies, *e.g.* agglutinins.

In concluding this outline of Madsen's lecture, I ought to add that he duly recognises the difficulties of his subject. "It cannot be denied that in spite of an enormous amount of work and a vast accumulation of facts, we still remain without the deeper understanding of some of the principal problems regarding immunity. This is, for instance, true of the question of the formation of the antibodies....The *negative phase* appearing in the curve representing active diphtheria antitoxin formation is still a mystery....It is just as difficult to find an explanation of the fact that this decrease in antitoxins is succeeded by a rise." I think these quotations will suffice to show that Madsen is careful to avoid any appearance of dogmatic finality.

Comment.

Madsen's explanation of his data is cogent and undoubtedly coincides with the views of the majority of immunologists. It is largely based on the assumption, which I admit to be the orthodox opinion, that serological tests furnish an accurate record of circulating antibodies. Still, this assumption is not absolutely proved to be correct; in some cases, if not with diphtheria antitoxin, serological reactions *in vitro* do not necessarily run parallel with immunological reactions *in vivo*. Therefore, as there are still some points about this antitoxin which have not been finally settled, it may be permissible to consider a different hypothesis, which is neither proved nor orthodox.

In the tissues and in the circulation there may be an antecedent form of that antitoxin which is demonstrable in the serum. This antecedent form may vary in its stability in different phases of the animal's immunological history, yielding more antitoxin in the serum when more stable and less when less stable.

On this latter view, the negative phase which is observed when a horse, already immunised or partially immunised, receives a fresh dose of toxin might be interpreted as follows. The new toxin is rapidly adsorbed by the cells which play a dominant part in the output of antibody. This union between toxin and the surface of the cell becomes firmer in the course of the first day (as in the case of foreign protein carrying anaphylactic antibody) and then causes a brief disturbance in the cellular mechanism, with consequent increase in the instability of the antibody which passes into the circulation. The result is a decrease in serological antibody but not a quantitative decrease in the less stable circulating antibody.

In the next phase, from about the third to the tenth day, the disturbance has subsided and the effect of cellular union with the new toxin is a quantitative increase of circulating antibody, accompanied by an increase of that proportion of it which will assume a stable condition in the serum.

After about the tenth day, the adsorbed toxin begins to be broken up and

this change in the cellular mechanism is marked by a diminution in the proportion of antibody which will become stable in the serum, though not necessarily by a quantitative diminution in circulating antibody.

This hypothesis seems to me rather less difficult to understand than the postulate that the same substance (the new toxin) can produce two opposite effects, first inhibition and then stimulation of the secretion of antitoxin.

The condition after the tenth day may be altered by non-specific means, their effect being, according to the above hypothesis, not to increase the total of circulating antibody but to modify its character, so that a greater proportion of it is capable of becoming stabilised in the serum.

This would involve the assumption that the catalytic action of a metallic salt, such as manganese chloride, can increase capacity for stabilisation, an explanation which seems to me less hazardous than the postulate that such substances actually stimulate the production of specific antitoxin *de novo*.

It has been shown by work on anaphylaxis that foreign protein can be adsorbed by the cells in one hour. This also appears to be the case with such substances as manganese or magnesium chloride, in the work recorded by Madsen. Proteins are highly complex and their interactions with the cell form a chain of events for the completion of which many days are required. With simple substances such as metallic salts the conditions are different. Here it is natural that the full catalytic effect of the reagent should be felt as soon as union with the cells is effected, and that it should diminish as soon as the salt begins to be eliminated from them. Hence the difference between toxin and metallic salts in their influence on the antitoxin curve.

Madsen states that the formation of antibodies has "practically ceased" on the ninth day after injection of toxin and that thereupon factors which destroy antibodies "have full play"; manganese chloride, however, will promptly renew the antibody content. It will be seen that the above hypothesis attempts to provide a different explanation. Perhaps further light will be thrown on the subject when these interesting observations on the action of metallic salts have been extended and confirmed.

The admittedly unorthodox view which I have put forward may be considered in relation to some of the puzzling facts concerning the co-existence of antigen and antibody. Why is it that toxin is neutralised by antitoxin *in vitro* and in passive immunity (and thereby loses its antigenic powers) but not in the circulation of an actively immunised animal? My suggestion is that in the last case the circulating antitoxin, unlike the serological antitoxin, is too unstable to form that firm union with toxin which would abolish the latter's antigenic function. It does not follow that the "active" antitoxin is indifferent to the toxin; it may form loose colloidal or chemical union with it, which would suffice to prevent the latter from exerting its full toxicity on the cells, and so would explain why an immunised animal is more tolerant of toxin than a normal animal.

I have a brief comment to offer on Madsen's secretory theory. If one starts

with (1) the view that antibodies are cellular secretions due to the stimulus of the specific antigens, one is inevitably led to consider in what way the antigens produce this effect. In such considerations, it seems to me, one cannot avoid the postulate that (2) the antigen must be brought into intimate contact with the cell, probably by adsorption, and must also modify the fluids which permeate the cell, probably by catalytic action. One might go on to amplify these considerations, but at this point I wish to call a halt. Hypothesis (2) is evidently contained within the wider hypothesis (1).

Why not try to economise hypotheses by starting with the smaller one (2)? It seems to me possible that considerable progress may be made with (2) alone. Perhaps it may not be necessary to encumber oneself with (1), which, apparently, would involve difficulties similar to those of Ehrlich's theory regarding the production of antibodies in excess of the cell's requirements.

Madsen's lecture raises another question about economy in hypotheses. If a stone is thrown into the air, it is controlled by the force of gravity during the whole of its course. But is there an equally valid law of nature which compels the animal body continuously to destroy the protective antibodies which it is laboriously manufacturing? Such rapid and continuous destruction would be something quite different from the normal using up of material (followed by its replacement) which is associated with living matter; and I should hesitate to agree that Madsen's postulate is a general law governing immunological processes.

Perhaps some simpler explanation can be found for the oscillations in the serological antibody curve during immunisation. It is known that the transition from life to death is soon followed by a very profound change in the body fluids. These serological antibodies are dead material; the living matter from which they were derived has undergone change, and many of its attributes have been destroyed. Instead of assigning the act of destruction to a constant and normal function of the living body, would it not be more plausible to explain it as being largely, though not entirely, due to the changes accompanying the transition from living to inert matter? This would lead to the less ambitious hypothesis, which I have suggested above, that the living material from which the serological antibodies are derived varies qualitatively (as regards capacity for becoming stabilised) as well as in quantity of antibody content, and that oscillations in the serological curve are partly attributable to the former type of variation.

LOCAL SUSCEPTIBILITY AND LOCAL IMMUNITY.

The main facts are the ordinary observations of clinical medicine. In most bacterial infections the clinical picture is an example of local susceptibility and local immunity; infections are distinguished from each other by the differences in the details of the picture, the distribution of the lesions, their characters, and their capacities for recovery.

The explanation of these differences is the task of pathology, and one part of the problem is to consider the functions of endothelium. How does this tissue, which is ubiquitous, participate in these manifestations of local susceptibility and local resistance? This question again must be subdivided into several, the one of immediate concern here being the question of endothelial participation in the local formation of antibodies.

As to the nature of such possible participation, it must be remembered that, though the endothelium of the blood and lymph channels may be regarded collectively as an important organ regulating the functions of the body, it is not an independent organ; its normal activities depend on the special characters of the adjacent tissues, and its capacities for dealing with bacteria also depend on local conditions. In any given locality, the endothelium, the fluids on either side of the endothelial barrier, and the tissues which depend on these fluids for their vitality act in conjunction as one mechanism. Thus there are local differences in endothelium, but, as these depend on environment, it does not seem likely that the characteristics of endothelium alone will fully account for the formation of antibodies; and for a similar reason it does not seem possible to regard cells of epithelial or other type as independent agents which are entirely responsible for the production of antibodies.

Some light on the clinical data, though perhaps not very much, is thrown by laboratory experiments.

It is customary in the text-books to quote a famous experiment by Wassermann and Citron on a rabbit. They injected typhoid bacilli subcutaneously into its ear, immediately applied a ligature to the base of the ear, and kept the ligature in position for several hours. After nine days they determined the bactericidal titre of the animal's serum and then amputated its ear. After this operation they found an immediate and rapid fall of antibody and inferred that the chief source of antibody formation had been removed. Many other experiments have been recorded which suggest that, in some circumstances, antibody is produced locally at the site where the antigen was injected. And this view is in conformity with the fact that failure has attended the numerous attempts to show that the general function of turning out antibodies is allocated to some central organ or tissue.

There is also laboratory evidence that the conception of local immunity must not be narrowed down to the idea that for the production of this condition some one type of cell, differing with different bacterial infections, is entirely responsible.

For example, if Shiga bacilli are introduced into the alimentary canal of a suitable animal, the intestinal mucosa is the susceptible tissue which is attacked. If, instead, the bacilli are injected into the peripheral circulation of a similar animal, the intestinal mucosa is again the site which is selected for attack; but in this case the bacilli must penetrate the capillary endothelium adjacent to the mucosa before they can reach the latter, and it is this endothelium which is, primarily, the susceptible tissue. If the above two experiments

are repeated on an animal which has been immunised by parenteral inoculation, both epithelium and endothelium of the intestinal area are found to be resistant. In all these events, as it seems to me, susceptibility or resistance must be attributed to the mechanism as a whole, not simply to a quality of one of its factors, epithelium, endothelium, or body fluids. And, though the susceptibility or immunity is local in the sense that it is manifested in the intestinal wall, it is not altogether local, since one of the factors in the mechanism consists of material derived from the general circulation.

Some further points of interest are raised by F. P. Gay. In a recent article on local and general immunity[1], he endeavours to distinguish "true local immunity" from mere evidence of the localisation in certain areas of antibodies derived from the general circulation, *i.e.* merely local mobilisation of a general form of protection. He defines "true local immunity" as an "Umstimmung" or "retuning" of the tissues, which causes them to react in a new fashion, and quotes some experiments with streptococci as affording evidence of this condition. A strain of fixed virulence was used and it was found that it regularly produced erysipelas in rabbits when inoculated intradermally in a dose of 0·1 c.c. The animals made complete recovery, although double the dose would produce fatal septicaemia. After recovery, the animals were completely immune against re-inoculation intradermally elsewhere on the body. But they were not protected against intravenous inoculation with the same dose, although "the minimal lethal dose is practically the same intravenously as is the symptomatic dose intradermally." Conversely, he found that "intravenous inoculation of sublethal doses protects the animal against intravenous inoculation but not against intradermal inoculation." Differences corresponding to these were found in the effects of intrapleural as compared with intravenous immunisation.

As regards dosage, these experiments illustrate a difference between unsuccessful and successful resistance which may perhaps be explained in this way. When the first intradermal or intravenous dose exceeds 0·1 c.c. the irritant action of the cocci ultimately breaks down the endothelial barrier, the result being generalised and fatal dissemination. With the correct immunising dose, this does not occur; the bacterial antigens are adsorbed on one or other side of the endothelium and cause this barrier to resist penetration by a larger dose which would be fatal to a normal animal.

One feature of the endothelial filter is that chemical and physical conditions on the one side of it (the lumen of the vessel) are not the same as those on the other side, which is in proximity to the tissues. Is this exemplified in the different effects of intravenous and intradermal immunisation? Since cutaneous immunisation at one site protects the whole of the animal's skin, the protective substances must get into the circulation, in order that they may be distributed throughout the skin area. Therefore it seems necessary to postulate two different kinds of protective substances, the one produced by cutaneous and the other

[1] *Journ. Immunol.* VIII. p. 1. 1923.

by intravenous injection. These differences are possibly attributable to differences in the character of the one or other side of the endothelial barrier which has absorbed the antigenic material. There is, however, an alternative supposition. In order to act as antigens, bacteria must be broken up, and the exact way in which they are broken up may depend on their particular location, *e.g.* in the skin or in the circulation; so it may happen that the antigens produced in the former situation differ, in some respects, from those produced in the latter, and that these differences are reflected in their antibodies.

Partial streptococcal immunisation may, in one of its aspects—immunisation against septicaemia without protection against erysipelas—bear a resemblance to some results obtained by Cecil and Blake in experiments on monkeys with killed pneumococcus vaccine. They found[1] that subcutaneous vaccination gave definite protection against experimental pneumococcus septicaemia, but did not protect against pneumonia produced by intratracheal infection. This limited degree of immunity they described as "humoral." The vaccine given subcutaneously evidently found its way into the blood stream; it may have modified the side of the endothelium facing the circulation, caused it to produce antibodies with particular characters, and increased its resistance to penetration by circulating pneumococci. In intratracheal infection, the lung would have to deal with pneumococci on the tissue side (which had not been immunised) of the endothelium.

The above examples prove nothing; and I do not think it would be possible, by adding to their number, to provide adequate data for the induction of any general principle. All that may be claimed for them is that they indicate the presence of many factors in local reactions to bacterial invasion and raise questions about endothelium without providing any definite answer[2]. After all, the clinical rather than the experimental data are the main facts requiring explanation.

Development of Hypothesis.

In the preceding sections I have endeavoured to prepare the way for the following elaboration of ideas about the capillary endothelium in relation to antibodies. If any hypothesis of this nature is worth considering, one wants to know more definitely what shape it is going to take.

Production of Antibodies.

The ordinary and admittedly vague idea of an antibody is that it is something (*e.g.* a precipitin or an agglutinin) which is produced in the animal body by the action of foreign protein (antigen) and reacts with that protein both *in vivo* and *in vitro*.

The current explanation of the way in which antibodies are formed is equally vague and tentative. It is commonly supposed that the stimulus of

[1] *Journ. Exper. Med.* xxxi. p. 519. 1920.

[2] For Besredka's views on local immunisation, his articles in the *Bull. de l'Inst. Pasteur* (xx. p. 473 and p. 513, 1922) may be consulted.

foreign protein causes certain of the animal's cells to protect themselves against injury by secreting something (the antibody) which acts as an enzyme towards this irritant foreign protein and digests it. There is no need to quarrel with this idea if it is taken merely as a suggestion and not as a literal statement of fact. There is certainly some resemblance between the stimulus of a foreign protein, introduced parenterally, and the stimulus which causes the secretion of enzymes by the digestive tract; and the action of an antibody on its antigen may, to some extent, resemble the catalytic action of a ferment. But it is very far from being proved that antibodies are ferments in the strict physiological sense of the term, or that they are primarily and essentially a cellular secretion comparable to the characteristic secretions of special glandular structures.

Hence it is not necessary to postulate that antibodies are the product of cells naturally endowed with a special secretory capacity; if it were, it would certainly be difficult to imagine why endothelium should possess this characteristic in preference to any other type of cell.

If, however, one thinks of antibody formation not as a special secretion but as a process which has something to do with filtration and results in a modification of the filtered fluid, then endothelium has a particular claim for consideration, because filtration is a specially important function of this type of cell.

Some idea of the delicate selective activity of a cellular filter may be derived from the work of Hamburger and his associates on the permeability of the glomerular epithelium of the frog's kidney[1]. They showed, by perfusion experiments with various sugars, that permeability or retention did not depend upon the size of the molecules but upon their precise stereo-chemical configuration. For example, glucose was retained but fructose and mannose passed through completely. Galactose was partially retained; the explanation of this was found to lie in the fact that this sugar, when dissolved in water, splits up into α and β forms which differ only in the relative positions of an H and OH group attached to an asymmetric carbon atom; the α form was retained, while the β form passed through. Two further points are worth recalling. Glucose was retained when perfused in the amount which is normally present in the frog's blood plasma; but, when it was administered in larger amount, the glomerular membrane became permeable. Again, when a minute trace of a foreign substance (0·004 per cent. of phloridzin) was added to the normal amount of glucose, the membrane at once allowed the glucose to pass through; after removing the phloridzin with pure Ringer's solution, the membrane was restored to the normal condition, *i.e.* it was completely impermeable to glucose when perfused in the correct physiological amount.

Obviously, capillary endothelium is a very different sort of filter from glomerular epithelium, and experiments with the latter give no direct information about permeability from the circulation into the tissues or in the reverse direction. But they help one to realise that the mechanism of endothelial permeability must be extremely delicate, that it reacts selectively to minute stereo-chemical differences in the body fluids, and that the introduction of very slight amounts of foreign material may bring about a profound change.

With this introduction, I may formulate briefly the following ideas about the endothelial mechanism in relation to immunity.

[1] *Lancet*, II. pp. 1039–45. 1921.

When foreign protein is introduced parenterally, some of it is adsorbed by the surface of endothelial cells and thereby modifies the character of the endothelial filter. In consequence, the fluids of the body which pass through this filter are also liable to modification. When the change in these fluids is found to be highly specific, it must be due to chemical as well as colloidal factors and must depend to a large degree on the precise stereo-chemical structure of the adsorbed antigen. The tissue fluids are highly unstable in their chemical structure; they form a loose union with the antigenic elements of the endothelial filter; this union is rapidly followed by dissociation, as the fluids pass through the filter; but it leaves on the filtrate a certain impression, *viz.* a chemical configuration which is adapted for union with free antigen under favourable colloidal conditions. In virtue of this change, the circulating fluids have become antibodies. Antibodies, then, may be formed in any locality where antigen is in colloidal union with an endothelial surface; they will continue to be formed as long as the endothelial filter retains this modified character imposed by the antigen; and they will tend to increase in quantity by extension of the area of modified endothelium. Some of these antibodies are probably very unstable and only exist in the circulation of the living body; others, however, such as the precipitins and the antitoxins, are relatively stable and are demonstrable in the serum obtained from the blood of an immunised animal.

There seem to be some advantages in this idea that antibodies are formed by passage of the body fluids through an endothelial filter which has adsorbed antigen.

(1) Ehrlich's theory was that antibodies are "receptors" (*i.e.* elements which unite with antigen) produced in excess by the tissues (which have entered into union with antigen) and carried over into the blood stream. But, on the current idea that antibodies are cellular secretions, it is difficult to understand why an antigen should stimulate a cell to produce "receptors" in excess and continually cast them off. One needs some explanation for the "excess." The idea of filtration seems to be helpful. The fact that fresh fluid is constantly passing through the endothelial filter would, if endothelium participates in the formation of antibodies, provide a simpler explanation of the production of a large amount of antibody by a small amount of antigen.

(2) Endeavours to allocate antibody production to certain special tissues have not led to satisfactory or concordant results, but rather suggest that antibodies may be produced in any part of the body. Why not in, or by the agency of, the endothelium, which is ubiquitous?

(3) This is not inconsistent with observations on localised immunity or local production of immunity, because there is reason to believe that the endothelium acquires special characters from the parenchymatous tissue which surrounds it.

(4) This hypothesis allows for the probability that, in addition to the well known and stable antibodies of the precipitin type, other and less stable

antibodies may be formed in the living body, *e.g.* antibodies which do not survive in the serum after bleeding an immunised animal or, at least, are not demonstrable by serological tests *in vitro*. It is also consistent with observations that specific substances which behave like antibodies may be formed very rapidly (unlike the ordinary antibodies) in response to a foreign stimulus.

It must, of course, be remembered that this hypothesis claims for the endothelial cell not more than a certain participation in the mechanism of immunity; it would be absurd to suppose that the functions of these cells could explain the whole of the mechanism.

The hypothesis is not of a purely chemical nature, as Ehrlich's theory is, but it allows for the specificity of chemical structure. It is not primarily an enzyme theory (cellular secretion of a catalytic agent), nor does it postulate the manufacture of a large number of ferment-like substances; but the endothelium may be assumed to exert a catalytic action on the fluids which pass through it, and these modified fluids may also possess catalytic properties.

The Nature of Antibodies.

For the purpose of many contributions to immunological work it is not necessary to raise puzzling questions, involving highly obscure problems, about the precise nature of antibodies. It suffices to know that an antibody is something which behaves in a particular way; if it is a precipitin or an agglutinin, precipitation or agglutination ensues when it is brought into contact with material containing the appropriate antigen; if it is an antitoxin, it neutralises the corresponding toxin; and so forth. And apparent difficulties can often be disposed of by pointing out that the antigen-antibody reaction depends upon colloidal conditions. For example, unfavourable proportions of the quantities of interacting colloids may explain "zone phenomena"; and the fact that colloidal union is often loose and may be followed by dissociation often removes any difficulty from the discovery that antigen and antibody may co-exist side by side instead of forming a new complex. In other cases, a third factor, alexin or complement, may be invoked as the necessary condition for completing an interaction between immune body and antigen. Furthermore, when other ready explanations fail, it is always easy to admit frankly that here is one of the unsolved problems of immunity; there is no likelihood that its solution, when discovered, will invalidate the safe dictum that "an antibody is what it does."

In relation to this comfortable attitude, there is nothing discordant, so far as I am aware, in the above suggestions about the participation of endothelium in the formation of antibodies; they are merely hypothetical and, whether right or wrong, would not affect current laboratory interests in the properties of serological antibodies.

At the same time there remain questions which are distinctly uncomfortable but cannot well be ignored, questions, for example, about the processes of natural or acquired immunity in the living body and the distinction between

specific and non-specific factors in resistance to bacterial invasion. In entering upon these, it is necessary to discuss the nature of antibodies more closely and the part played by the capillary endothelium may assume a more definite importance.

The position bears some resemblance to the much discussed question about the nature of alexin. Nobody has succeeded in explaining what alexin really is. In ordinary laboratory routine this causes no difficulty; alexin, generally in the form of fresh guinea-pig serum, simply is "what it does"; and its capacity for doing certain work is very distinctive and can be estimated quantitatively with a high degree of accuracy. But some enquiring spirits, whilst accepting these facts, are not satisfied; they are not prepared to accept the inference that the work attributed to alexin is really due to the action of some special substance which, sooner or later, will be isolated by the biochemists and equipped with its chemical formula; they think that the action in question is attributable rather to the interplay of labile constituents in the serum and that all attempts to isolate and purify a special substance will lead to the destruction of "alexin" and will therefore defeat their own object.

Similarly with antibodies, one may start with the conception of a complex unit, of protein origin, which is chemically equipped so as to "fit" antigen. This is obviously true of the antibodies contained in an immune serum and may be demonstrated both *in vitro* and by appropriate animal experiment; and the amount of such antibody may be titrated with much precision. But is it safe to argue back from these serological data to the conditions which obtain in the living body of the naturally resistant or the actively immunised animal, and to postulate that in the latter circumstances the defensive mechanism consists, in part, of chemically distinct units identical with the special substances which are assumed to exist in an immune serum? Two questions have been raised about alexin. Does the work which is attributed to it in the test-tube correspond to work which is carried on in the living body? Is it a special substance? And about antibody, though differing from alexin in its greater stability *in vitro*, two similar questions may be asked. Does the living animal possess antibodies which are identical with serological antibodies? Is each different antibody reaction attributable to a different and special substance?

These questions involve various considerations, some of which are discussed in the following paragraphs.

Specificity of Antibodies.

Though it is convenient, and often quite legitimate, to distinguish "specific" from "non-specific" factors in immunity, it must be remembered that these terms are not always sharply separable and that neither is strictly appropriate to some of the events which take place during infection and resistance. For example, there is a colloidal balance between the fluids within the capillaries and the tissue fluids on the other side of the endothelium. When bacteria or

other foreign substances are introduced parenterally, this balance is disturbed and tends to be readjusted to a new level. The new level, in the adjustment of which the endothelium participates, is not "non-specific," since it differs according to the nature of the disturbing factor; on the other hand, one cannot go so far as to say that every different disturbing element tends to be followed by a correspondingly different (*i.e.* "specific") readjustment of colloidal equilibrium.

Similar considerations apply to the conception of antibodies as being either "specific" or "non-specific"; in some cases it may not be appropriate to use either term. The extremely delicate specificity of the precipitin reaction to foreign protein by no means justifies the assumption that there is a special antibody for every antigen, the former being "specific" when produced by immunisation with its particular antigen and "non-specific" when found in an animal which has not received this treatment. The normal constituents of plasma react in many ways to foreign protein which they encounter; and then, after this protein has produced some modification of the endothelium or other tissues, they tend to settle down to an equilibrium which may be somewhat different from their former equilibrium. In virtue of this change, when they again encounter this foreign protein their second reaction with it may differ, in some respect, from the first; but it is, in the main, the same type of reaction; the respect in which it differs does not justify a fundamental distinction between the reaction of an antibody (the second reaction) and the former reaction of the normal plasma, supplemented, where requisite, by the arbitrary postulate of normal or non-specific antibodies. In other words, what is currently known as an antigen-antibody reaction may be regarded as no more than a particular phase of a more general reaction between the constituents of plasma or serum and foreign protein.

According to this view, the conception of "antibodies" has to be widened. They are really much more than a particular chemical group (attached to a protein vehicle) which will "fit" a particular chemical group in the molecules of the antigen[1]. It is also implied that they are of variable stability and that a distinction must be drawn between the highly unstable factors in immunity, which are inseparable from vital activity, and the relatively stable factors which may be demonstrated by serological tests. The real "antibodies" are the constituents of plasma as they exist in the living body; and their real mode of action is part and parcel of the ceaseless succession of interactions which is characteristic of living matter. In serum only remnants of them are left, *viz.* those remnants which have survived the changes from living to inert matter. It is suggested, further, that widening the range of "antibodies" in this way may be one means of satisfying the requirement for the discovery of "new kinds" of antibodies.

"Antibodies," then, are not mere counterparts of antigens, but are of a

[1] When using the word "antibody" in this wider sense, I write it with inverted commas, to distinguish it from the restricted sense of the term which is firmly fixed in immunological literature.

quite different nature and are much more complex in their action. Their activities are those of a living mechanism and include the functions which are often (erroneously, in my opinion) attributed to special entities called complement or alexin. Antigens, on the other hand, are simply dead foreign protein, capable of being broken up in various ways; they certainly influence the living mechanism, but they are not an "active" part of it, in the sense in which circulating plasma or even fresh serum is "active." And this difference still holds good if, as I think is permissible, the conception of antigen is widened so as to include the varying products of metabolism which may occur in bacterial infections and may act antigenically, *i.e.* may lead to special (or specific) changes in the cells and fluids of the body. The real "antibodies" exist in the normal animal, whether resistant or susceptible, before the introduction of antigen; the antigen does not create them, though it modifies them in the case of the susceptible animal.

The conception of "antibodies" as a living mechanism in the circulating plasma differs from the purely chemical idea of them as molecules to which particular chemical groups are attached; this difference is involved in the view that in the change from life to death only the more stable functions of antibodies survive, *viz.* those functions which are not dependent on the ceaseless changes associated with metabolism.

The above ideas about antibodies and equilibrium are not new. I am not competent to correlate or compare them with Landsteiner's theory of electro-chemical affinities, but they seem to me to bear at least a rough resemblance to the following postulates which that author formulated many years ago[1]. (1) An antibody may react with a large number of other substances which it encounters, the reaction with the homologous antigen being no more than a special or distinctive instance; it is superfluous to suppose that there is a chemical group of a particular configuration to correspond with each of the numerous reactions of an immune body. (2) The tissues of the normal, non-immunised animal have a capacity for uniting with colloids of widely different characters; this conception should replace the assumption that there are special receptors in each individual case. (3) An equilibrium is maintained between the colloidal components of one and the same animal body; and the disturbance of this condition through the introduction of a foreign colloid leads to new formation of immune bodies. (4) Antibodies form a series in an ascending scale of specificity. (5) Immune reactions are related to non-specific processes of adsorption.

This view also suggests a way of endeavouring to link up natural immunity with acquired immunity. Though there are obvious differences between the two, they have certainly much in common and it is highly improbable that they depend on entirely different mechanisms. It may, however, be a mistake to try to explain natural immunity in terms of acquired immunity. Why not try the reverse order of procedure?

In animals which are naturally immune against a particular bacterial infection, it is clear, at least in some cases, that antibacterial substances are present in the circulation, though, when the animal is bled, they are not usually demonstrable in the serum, even if this is revived or reactivated by the addition

[1] *Zeitschr. f. Immunitätsforsch.* Orig. IX, p. 779. 1911.

of alexin. Their disappearance does not justify the assumption that they are something quite different from "antibodies"; they may be labile "antibodies" which disappear, as such, on withdrawal from the living organism.

In the next place, the various species of animals which are susceptible cannot be lumped all together in sharp contrast with the naturally immune. On the contrary, the former all possess natural powers of resistance in greater or less degree and, in relation to a given type of bacterium, might be arranged roughly, according to their species, in a descending scale, commencing with the very highly resistant which are not far removed from the naturally immune, and progressing by easy stages to the very slightly resistant, in which high susceptibility is the more conspicuous feature.

In acquired active immunity, throughout the entire range of the susceptible groups, the main and primary fact may be regarded as a modification or reinforcement of the animal's natural antibacterial substances or true "antibodies," which, in the naturally immune animal, do not require such reinforcement. This change may or may not be followed by a secondary event, the appearance of serological antibodies. Their appearance, however interesting and important in other respects, should not cause one to lose sight of the primary event.

The Activities of Antibodies.

This view of the mechanism of "antibodies" may be developed a little further. Their activities may be regarded as presenting a variety of phases which should be considered one at a time.

(1) The normal constituents of the plasma are "foreign" in relation to any alien protein which is introduced parenterally. They may not react with it at all, as when the protein (or some of it) persists for a variable period in the circulation and is then excreted, unaltered, in the urine. But if, as is more commonly the case, they do react with it in one way or another, they are behaving as "antibodies." The most important example may be taken to be the way in which living bacteria are disposed of by the naturally immune animal. The normal plasma constituents interfere with the vital processes of the bacteria (by producing alterations in surface tension, in assimilation of food, in capacity for reproduction, or by other means) and their destruction, often completed by phagocytosis, is the result. This interference may be regarded as due to labile reactions between plasma and bacteria (or bacterial products) with the repeated occurrence of loose union followed by dissociation. In the end result, whilst the bacteria are disposed of, the plasma constituents have not been changed. This I regard as the primary and most important feature of "antibody" action. The "antibody" is in existence before the antigen is introduced and remains unaltered after the antigen has been disposed of.

(2) In the susceptible animal, the normal "antibodies" cannot accomplish this task unaided. Certain characteristic elements of the foreign protein (disintegrated bacteria or other non-living material) are adsorbed by the tissues,

particularly by the endothelial cells. The consequent modification of the endothelial filter causes modification of the fluids which pass through it. Hence the plasma constituents become better adapted for forming loose union (followed by dissociation) with the foreign protein; when this protein is a living bacterium, they become better adapted to interfere with its vital mechanism. This modification in the properties of the original plasma constituents provides for the second aspect of "antibody" action; it is something which is "acquired," not as a new and independent mechanism but as a reinforcement of the natural mechanism. At the commencement of this phase the "antibodies" are still labile and are not demonstrable in the serum.

(3) In the next stage, the "antibodies," as they exist in the circulation, behave as before; their relations with antigen are still those of loose union and dissociation and do not result in a firm adsorption compound; but the change in the original constitution or balance of the plasma constituents is of a more permanent nature, with the result that the acquired affinity for the foreign protein survives in the serum and there becomes stabilised. This is the stage when the animal's serum may give a precipitin or other reaction *in vitro*, *i.e.* the stabilised antibody can now form a relatively firm adsorption compound with the antigen.

(4) If a disturbing factor is introduced, *viz.* reintroduction of the same antigen, there may be a partial reversion from stage (3) to (2). Is this to be explained on the ground that the labile "antibodies" are now given fresh work to do, in forming loose union and dissociation with the new antigen, and thereby lose some of their tendency to stabilisation, as is manifested by a temporary drop in the titre of serological antibody? This may be some part of the explanation, but probably the more important influence is the disturbance in the endothelial filter, caused by adsorption of new antigen, and followed by temporary increase in the instability of the "antibody" passing through into the circulation. The disturbance is only temporary and is soon followed by a return to stage (3), with perhaps increased output of antibodies demonstrable in the serum.

(5) After stage (3) has persisted for some time (up to the peak of the serological antibody curve), there is again a partial reversion to stage (2), without the introduction of any disturbing factor. This change is associated with the disappearance of the adsorbed antigen.

(6) Though the antigen has disappeared, the endothelial filter continues to turn out "antibodies" and these may be rendered less unstable (leading to a renewal of stage (3) with perhaps a still higher peak to the antibody curve) by the introduction of a non-specific influence. A good example of such an influence, according to Madsen, is manganese chloride, the assumption being, on my hypothesis, that, when this salt is adsorbed by endothelium, its catalytic action on the fluids which pass through diminishes their instability.

(7) The persistence of the capacity to form "antibodies" after the disappearance of the antigen varies according to the nature of the antigen; the

duration of immunity towards different infections ranges from a few weeks to a life-time. On what do these differences depend? On the capacity of certain cells (? endothelial filter) to renew, during metabolism and reproduction, that particular chemical structure which, by catalytic action, invests the filtered fluids with the properties of "antibodies."

(8) Continued production of "antibodies" over a long period means continuance of a modification (which may vary in degree) of filtered fluid. It is a qualitative conception, not quantitative, *i.e.* it does not mean that a certain quantum is manufactured on one day, a fresh quantum on the next day, and so on. Hence there is no need to imagine that the body is in danger of getting drenched with an "excess" of "antibodies," or that it has to save itself from this peril by constantly destroying them. On this view, there is no need to postulate that serological antibodies, even if they exist as such in the circulation of the actively immunised animal, are constantly undergoing destruction, in the way suggested by Madsen.

(9) The hypothesis that, in the change from living plasma to serum, "antibodies" lose their instability and assume the serological type does not exclude the assumption that there is also a tendency to this stabilisation in the living body, particularly when the "antibodies" are not freely circulating but become adsorbed to the surface of cells. Such adsorption and stabilisation, when it leads to the precipitin type of antibody, is the predisposing cause of anaphylactic shock.

Perhaps these considerations may help to explain some of the differences between reactions *in vivo* and *in vitro*. For example, serological tests may show that, in active immunity, apparently unaltered antigen may circulate for a long time in the living animal together with free antibody. Why does not neutralisation take place in the form of a precipitin reaction? It may, perhaps, be said that colloidal conditions in the living body differ from those in the test tube and are unfavourable for such a reaction; but this can hardly be the whole of the explanation. Though the antibody demonstrable in the serum was of the precipitin type, the "antibodies" actually circulating may not have been in a sufficiently stable condition to bring about this antigen-antibody reaction. There is a further point about the recovered antigen being apparently unaltered. Yes; unaltered in the sense that it is still a precipitinogen, but not necessarily unaltered in other respects by the circulating "antibodies." This last distinction may be of no particular interest when it is merely a question of identifying dead foreign protein by a specific reaction, but it may be of the greatest importance when the circulating "antibodies" are acting upon living bacterial protoplasm; they may be producing profound changes in the latter without destroying its "hall-mark" as specific antigen. Furthermore, one must accept the view that, in the course of immunisation, both antigen and antibody are constantly being adsorbed by fixed tissue cells, and must, therefore, frequently come into contact with each other in such situations. Then why is not immunisation an unfortunate reiteration of anaphylactic shocks?

One reason probably is that the adsorbed "antibody" is not stabilised into the precipitin type.

So far, I have been considering "antibodies" in relation to active immunity. There are obvious differences in passive immunity, *i.e.* where the serological antibodies are transferred to a non-immunised animal. In such cases, the antibodies are already stabilised. Theoretically, they may act in one or more of three different ways: (1) they may remain temporarily in the stable condition, become adsorbed by bacteria or bacterial products, and enter into antigen-antibody combinations which are similar to those demonstrable *in vitro*; (2) they may break up into unstable "antibodies" behaving like those originally present in the circulation of the immunised animal; (3) they may be adsorbed by endothelium and modify the endothelial filter, thus acting like antigen specially prepared so as to produce immediate formation of "antibody." The possible importance of methods (2) and (3) lies in the fact that the efficacy of therapeutic sera, with the exception of antitoxic sera, cannot usually be explained by (1) alone; for example, the utility of anti-anthrax serum may be largely due to (2) or (3). And deficiency in these two properties may be the reason why many sera are of little therapeutic value though they are well provided with serological antibodies.

Doubts and Conclusions.

Doubts.

Observed facts about the ways in which antigens and antibodies manifest their activities are generally recorded on the assumption that it is sufficient to define these substances in terms of what they are actually found to do. But sometimes it is desirable, in the interests of progress, to press for a closer definition and to ask for something more detailed than the statements that antigens produce antibodies and antibodies "fit" antigens. Then the trouble begins; one has now passed from the region of solid fact to that of tentative theory, where it is necessary to introduce physiological conceptions which are much vaguer than the concrete terms of chemical and physcial reactions.

That is not the whole of the difficulty. If, in exploring a country, one comes to cross-roads, it is necessary to decide on the route to take. It may be possible to assure oneself that all the roads save one run in the wrong direction; then the one exception is clearly to be selected; it is the best working hypothesis. And if, at each point where the tracks diverge, one can come to an equally clear decision, there will be the satisfaction, at the journey's end, of knowing that every effort has been made to travel in the right direction. But if, at each of these successive cross-roads, it is impossible to exclude all routes but one, then the traveller has to admit, at the end of his journey, that his course has largely been determined by an element of chance or by his personal equation. He is open to the criticism that other persons, with equal justification (or lack of justification) would have mapped out quite different courses; and about one

and all of such explorers there will be added the remark that their exertions have been "merely speculative."

I can exemplify some of these "cross-roads" in the subject I have been discussing.

(1) It has often been said that knowledge of acquired immunity will not make rapid progress until more is known about natural immunity. Experimentally, the former kind of immunity is obviously easier to investigate, but it leaves the latter unexplained. One may start, however, with the view that acquired immunity is simply a reinforcement or readjustment of the mechanism of natural immunity. This hypothesis appeals to me as probably containing a considerable element of truth, and I have endeavoured to follow it up. Another person might choose quite a different course. He might say that the stimulus of infection brings into play a new mechanism, a factor which supplements the normal machinery but differs from it and is of independent origin, and that, therefore, acquired immunity cannot be explained in terms of natural immunity. I am unable to prove that this line of thought is devoid of justification, though I have preferred the alternative route.

(2) If natural immunity holds the clue to acquired immunity, then, as acquired immunity appears to be largely an affair of antibodies, the mechanism of antibody production must pre-exist in the normal animal, whether susceptible or immune. The adoption of this view leads, as I have endeavoured to show, to a wider conception of "antibodies." But there are alternatives which cannot be lightly dismissed. For example, it has not actually been proved that antibodies, of one kind or another, really are the main element in the normal defensive mechanism; it may be of quite a different nature.

(3) It is quite clear that the ordinary known serological antibodies do not suffice to account for either natural or acquired immunity (in their humoral aspects), and the distinction I have attempted between stable and unstable conditions of antibodies may help to account for this. But there are other possible explanations. Different kinds of antibodies may exist, though not yet discovered; or, again, it might be argued that the facts to be accounted for are not attributable to antibodies but to some other factors. For example, when the serum of a naturally immune animal is found not to be antibacterial, alternative hypotheses may be brought forward in place of the suggestion that the antibodies in the living plasma are unstable and perish in the serum.

(4) Endothelium has not been proved to be the site of antibody production. If, as is quite possible, this function really resides in some other types of cells, another explorer's route may diverge widely from mine.

I think the above examples are enough to make it clear that I have no illusions about the difficulty of pursuing the subject I have chosen with a coherent thread of argument.

Conclusions.

The most obvious feature about antigen-antibody reactions is their precision. They afford a constant reminder that the antibody must contain some special chemical component which exactly "fits" the antigen. But the number of possible antigens with which the animal body may have to deal, when foreign protein is introduced parenterally, is practically unlimited. This fact is illustrated in the text-books, which generally quote some colossal figure giving the number of different ways in which it is possible to combine the 20 amino-acids or "building stones" into which proteins can be broken up. It would seem, then, on the "lock and key" hypothesis, that there must be available in the animal body an equally colossal number of "keys." I see no particular reason to disagree with this line of thought. It is sufficient to point out that it leads to nothing; it merely implies that there is no cause for surprise, whatever may happen, all that has occurred being a particular rearrangement of "building stones."

The futility of attempting to identify innumerably different arrangements of "building stones" makes one realise that, in studying antibodies, one cannot be content to regard them as mere counterparts of antigens. To modify the metaphor and call antibodies "master keys" may be welcomed as a slight departure from the "counterpart" idea, but I do not think it leads very far. The main defect of the "lock and key" conception is that it gives no clue to the method and order which regulate the body's activities. That is what the immunologist wants—a physiological explanation of the way in which the animal organism produces antibodies. When he has obtained that, it may be taken for granted, without the necessity for chemical analysis, that the "keys" or "building stones" are arranged as they should be.

By a physiological explanation I do not mean the simple device of describing antibodies as enzymes which are secreted by cells of the body. That is often merely a change in nomenclature which is no more explanatory than the "lock and key" idea. Each antigenic variant of foreign protein stimulates certain cells of the body to produce a different and special kind of enzyme which acts as an antibody; this implies that the body is capable of producing an indefinitely large number of different ferments. As antibodies bear some resemblance to enzymes, one might give a qualified assent to this statement. But what is it going to lead to? To call antibodies "enzymes" does not produce order out of chaos; it merely postulates that a certain physiological function may be exercised in an infinite variety of ways. What is wanted is some idea of an orderly mechanism of antibody formation; when that is acquired, the minor question of a resemblance to enzymes may be discussed at leisure.

It is known that, as a result of immunisation, something is present in the serum which was not there before; and it is safe to conclude that the serological change is referable to some event which has occurred in the living body. But one cannot make the converse assumption that changes occurring *in vivo*

during immunisation are necessarily represented by demonstrable changes in the serum. On the contrary, as there are many events in immunity and infection not associated with the presence or absence of serological antibodies, it seems more natural to assume that some of the properties of the circulating antibodies have been changed or lost in the transition from living plasma to inert serum. And, unless cause can be shown for ruling it out of court, I think this latter assumption should be tested as a working hypothesis in the physiological explanation of antibodies.

How does the foreign protein act as a stimulus? Various experimental data lead one to think that the first event is adsorption of some of the protein constituents by the surface of certain tissue cells. *But what is the next step?* That is the difficulty. Definite proof is lacking and it is necessary to select from alternative hypotheses. Is it to be said that the adsorbed protein causes the cell to become a special kind of chemical laboratory which proceeds to turn out antibody? Before agreeing that this is the best explanation available, I think it is worth considering whether filtration may not play some part in the process. It seems to me that this is plausible, particularly if one supposes that the capillary endothelium acts as the filter.

With reference to the earlier stages of antibody formation, two hypotheses which I have put forward are (1) that the properties of the endothelial filter are changed by the adsorption of foreign protein and (2) that antibodies are formed by the modification of the fluids which pass through this altered filter. But antibodies may still be turned out at a later stage, when the adsorbed foreign protein has disappeared. To account for this, a further hypothesis is necessary, (3) a persistence, in the normal constituents of the endothelium, of some impression which was formed in the earlier stages. The result of the "impression" is persistence (for a period of variable duration) of the modified character of the filter; the nature of the "impression" is assumed to be some alteration in normal metabolic processes, an alteration which may be passed on to new generations of cells.

The above are examples of the working hypotheses which I have attempted to develop in the preceding pages. I do not claim that they are necessarily the best; out of the many possible alternatives, probably other persons who may be interested in this subject will find that a more helpful selection might have been made. My main contention is that more attention should be paid to the development of a physiological conception of antibodies.

(*MS. received for publication* 1. XII. 1923.—Ed.)