THE NATURE OF ANTIBODIES.

By ARTHUR EASTWOOD, M.D.

From the Pathological Laboratory of the Ministry of Health.

CONTENTS.

STRICTLY DEFINED ANTIBODIES.

CONFUSION will be avoided if attention is first focussed upon antibodies which conform to the original definition, as being definitely attributable to an antigenic stimulus, with postponement of questions about other properties of plasma or serum which may bear greater or less resemblance to such antibodies. This definition may be expressed as follows: an antibody is a new combining property which is found in the serum after immunisation and is usually specific for the antigen used for immunisation. Whilst a beginning must be made by a statement expressed in these or some similar terms, it is obvious that the see-saw method of defining antibody and antigen in terms of each other gives no insight into the nature of antibodies.

Origin.

What is the mechanism of the antigenic stimulus or influence which is responsible for antibody formation?

In the first place there are two sharply opposed hypotheses which, in my view, should be discarded. (1) The first is Ehrlich's. All antibodies are already pre-existent within some of the cells of the animal to be immunised; the antigen stimulates these cells to produce them in excess and those not required for the cell's maintenance are turned out into the blood stream as circulating antibodies. This is an arbitrary assumption which is devoid of proof that any acquired antibodies are pre-existent as intracellular substances; and it is still more arbitrary to apply it to all antibodies, by imagining that an animal's

https://doi.org/10.1017/S002217240001857X Published online by Cambridge University Press

cells are already equipped with each antibody which immunisation with one or other of innumerable antigens may evoke. (2) The antigen does not act as a cellular stimulus. It combines directly with the blood-proteins and the product of combination is the antibody, which thus contains an element of antigen. This view is contrary to elementary facts: (*a*) a minute quantity of antigen may produce an amount of antibody which is so large that it would be impossible for each antibody molecule to contain a portion of antigen; (*b*) there may be renewed reproduction of antibody without the introduction of additional antigen; (*c*) when antigenic specificity has been changed, *e.g.* by linkage of protein with atoxyl, it has been shown that the "atoxyl specific" antiserum is free from arsenic[1].

Then there is (3) the hypothesis which is a sort of compromise between (1) and (2). Antibodies are new cellular secretions due to ingestion of antigen, some element of which is incorporated in these secretions and gives them their specific character. I think this view is open to the same objections as (2).

Now I come to a thesis which affords a useful starting-point, because it may be worded in general terms which will meet acceptance from the majority of immunologists. The antigen must in some way act as a cellular stimulus which causes a change (Umstimmung) in the activities or reactive capacities of the animal's cells. Here is a fairly broad basis for agreement. When, however, one asks for some detailed explanation of the nature and mechanism of this Umstimmung, one is again confronted with widely different opinions. It would take too long—and I doubt if it would be profitable—to analyse them seriatim; so I think the best method of criticism is to raise a question which involves them all. Are antibodies cellular secretions? I will first say something in support of this view.

It may be assumed that the first stage in immunisation is adsorption of antigen by the surface of the cell which is to produce the antibody. Hence there is a change in the selective permeability of the cell, with a consequent difference in the characters of the material which passes into the interior and is there dealt with by the cell's synthetic activities. It follows that there is a difference in some of the products which the cell elaborates and turns out into the circulation; in particular, analysis of the blood will show that the globulin fraction has now acquired the property of an antibody. This initial change in the cell's method of synthesis becomes an acquired characteristic which constitutes the cell's Umstimmung. The persistence of the Umstimmung beyond the period when adsorbed antigen was present is sufficient indication that there is a real readjustment of cellular activity which is not simply an assimilation of antigenic material.

The change in the cell, it may be suggested, is comparable to bacterial adaptation to a new environment. For example, a pneumococcus which was originally of Type I may, after growth in the presence of dead Type II, be converted into II; it now secretes II soluble substance, which it never secreted before; and this habit is perpetuated after removal from

[1] Berger and Erlenmeyer, *Zeitschr. f. Hyg.* **113**, 79, 1931.

the environment which contained dead II material. Similarly, the animal cell has learnt to secrete something new (an antibody) without further aid of the stimulus (the antigen) which led to the production of this changed capacity.

There is a further question of interest about this secretory hypothesis. Does the antigen, perhaps after some preparation on the surface which has adsorbed it, actually pass into the interior of the cell? There are two main alternatives. (*a*) The antigen, being foreign protein, would seriously damage the cell if it penetrated into the interior; the change which it produces is confined to an altered permeability of the surface; this changes the character of the material (non-bacterial) which is received for synthesis within the cell; the changed secretion is thus a secondary event, the modification of the surface being the essential Umstimmung. (*b*) Surface adsorption of antigen is followed by a second stage, penetration of antigenic material into the interior of the cell. In the attempt to assimilate it, the synthetic capacities of the cell are changed and the change persists after the disturbing antigenic material has been excreted, the new synthetic product being purely of animal origin. Thus the essential Umstimmung occurs in this second stage; it is a change in the mechanism of synthesis rather than in the products presented for synthesis, just as a change from Type I to Type II pneumococcus may be ascribed to a change in the mechanism of synthesising a different soluble substance out of the same animal material.

On my own view, which I first outlined in 1924[1], I admit that antibody formation involves a change of reactivity (Umstimmung) on the part of certain tissue cells, but I do not agree that antibodies are chemical secretions manufactured by particular cells and then turned out into the blood stream. I do not regard them as independent chemical entities but think that the idea of "secretion" should be replaced by "filtration," which is much simpler and removes the difficult postulate that the cells of the body are called upon to acquire an extremely wide range of new synthetic capacities. Antibodies, then, are chemico-physical modifications of the plasma produced not by direct interaction between free antigen and plasma but by filtration of the plasma through capillary endothelium which has been modified by adsorption of antigen and retains this modification (Umstimmung) after the antigen has disappeared. By ascribing this importance to endothelium, whilst rejecting its supposed secretory capacity (reticulo-endothelial theory), I propose to reconcile the opposing views of those who affirm and those who deny that antibodies are products of cellular activity. And, on my view, antibody formation is not in any way associated with the phagocytic capacities of fixed tissue cells; my hypothesis is emphatically not a rehabilitation of Metchnikoff's doctrines.

Specificity.

Ehrlich's work, subsequently elaborated by Landsteiner, on the extremely delicate chemical specificity of antibodies must be accepted as the foundation of immunology and should be sharply distinguished from the doubtful doctrines of the Ehrlich school about the production of antibodies and the nature of their reactions with antigens. How is this specificity to be explained?

It may be agreed that the first stage of the Umstimmung in immunisation

[1] The capillary endothelium in relation to antibodies, *J. Hyg.* **22**, 355, 1924.

affects the surface of the cells responsible for antibody formation. Then those who insist on a secretory hypothesis, whilst admitting that antibodies are not pre-existent, must proceed to postulate further changes within the interior of the cell in order that the elaborated product may be specific. On my view, which substitutes "filtration" for "secretion," the change in permeability of endothelium can account for specificity without the need to assume that the interior of these cells, as well as their surface, is radically altered. In the following paragraphs I make an attempt to visualise the process.

First as to the general characters of a combining affinity which behaves as a specific antibody. In a recent paper on "Combining affinities"[1] I discussed the newer conception of cellular enzymes which regards them not as so many discrete and separate entities but as an elastic and variable array of "centres of activity." Thus, out of the many side-chains, *a*, *b*, *c*, *d*, etc., which are attached to protein, chemico-physical conditions may determine that *abc* act conjointly as a combining centre or enzyme; under different chemico-physical conditions this centre may be disintegrated and another centre, *adf*, may be formed, which exhibits a different mode of enzyme action; and so on. This conception need not be confined to enzymes but may be utilised in general terms to explain other readjustments of protein combining affinities, *e.g.* the cellular Umstimmung produced by antigen. The same conception may also be applied to the constituents of the circulating plasma. An antibody may be regarded as a combining centre, *abc*, which is not present in the normal plasma but is produced by a readjustment of the pre-existing units or side-chains, *a*, *b*, *c*, *d*, etc.

Then why is *abc* specific? On my filtration hypothesis, the endothelial filter which has adsorbed antigen has acquired a new combining capacity, X, which is characteristic of the antigen. The effect of filtration is that X makes loose combination with certain plasma constituents. This is followed by dissociation; X remains *in situ* and the plasma passes through the filter. But the plasma has been altered by contact with X; it has formed a new combining centre, Y.

The simplest way of picturing the origin of X and its relationship to $\bar{Y}$ is to recall elementary data about organic compounds, *e.g.* tartaric acid, possessing asymmetric carbon atoms. There is a *dextro*-rotatory and a *laevo*-rotatory form and the two may combine into the racemic form which is optically inactive by external compensation. Here one may refer to Landsteiner, who has shown[2] that, when the tartaric acids are made antigenic by linkage with protein, their stereo-chemical configuration determines the specificity of the antibodies produced.

One may assume, then, that the antigen possesses a specific combining group which is in (say) the *d*-form and finds on the endothelial surface a complex due to the union of *d*- and *l*-forms. These it breaks up, uniting with *l*- and leaving *d*- as an endothelial property. This is the X, the combining capacity, acquired by the endothelium, which is characteristic of antigen. A similar conception may be applied to modification of the plasma by filtration. The endothelial *d*- combining centre finds in the plasma a complex of *d*- and *l*- which it breaks up, the first step being temporary union with *l*-; this is followed by dissociation as the plasma passes through the filter. The filter remains unchanged, as its *d*-form is firmly incorporated in the endothelium; but the plasma now has free *l*-combining centres. These are Y, the new combining centres of the plasma which are the counterpart of X. Thus the specificity of X and Y may be regarded as due to their being *d*- and *l*-forms which are mirrors of each other.

These two forms do not make permanent union *in vivo*, because one of them is firmly

[1] *J. Hyg.* **32**, 301, 1932. [2] *J. Exp. Med.* **50**, 407, 1929.

incorporated in the endothelial surface. But the antigen, which also possesses the *abc* type of combining centre in the *d*-form, may, when not prevented by adverse colloidal or other physical conditions, combine with the *abc* of the plasma or serum (the *l*-form) after the analogy of production of a racemic form by union of the *d*- and *l*-forms. Such union is exemplified in the ordinary serological reaction between antigen and antibody.

This, of course, is no more than a crude and diagrammatic way of visualising a method in which an antibody may be produced as a chemical "counterpart" to the specificity of an antigen. What actually goes on in the reactions of living tissues and plasma must be much more complex. Still, though vitalistic phenomena defy analysis, there is a reiterated sameness about the antibody response to every variety of antigen which suggests an automatic similarity in the chemical part of the mechanism. If this aspect of immunisation can to some extent be visualised, one gains something which is definitely more concrete than the vague statement that an antibody is formed in response to a specific stimulus. I may add that the antibody, according to my suggestion, is not pre-existent (as in Ehrlich's view) but is formed by the splitting up of a pre-existent organic complex. Possibly some of Ehrlich's staunch supporters might accept this alternative as involving no more than a slight modification of this part of Ehrlich's doctrine.

Irregularities of antibody formation.

Precise specificity, with the formation of a chemical "mirror" or counterpart of antigen, is the most striking feature of antibody formation; but it is by no means the invariable rule.

The irregularities of special interest are those which are evidently due to animal idiosyncrasies, when the antigen is not a mixture but sharply defined and relatively pure. After injection with such material, individual rabbits may vary very considerably and satisfactory results may be obtained from only one or two out of several animals. Horses, again, differ from each other and it may also be found that the antibody output from a horse is not quite the same in character as that from a rabbit. If this animal "individuality" could be satisfactorily explained, knowledge of antibody formation would have made considerable advance. At present one can only make tentative suggestions of possible reasons.

Is the defective animal devoid of some material upon which the mechanism of antibody formation depends? For example, do five out of six rabbits fail to possess the particular organic complex (present in the sixth rabbit) which can be dissociated so as to yield a precise counterpart to the combining affinity of the antigen? Or, if rabbits give a better output of a particular antibody than horses, does this mean that horses are deficient in some particular complex?

There are difficulties about an affirmative answer to these questions. One would rather assume that the substance in question is widely distributed in the animal kingdom and not limited to particular species or individuals. And

this assumption has not been proved to be wrong. If comparative biological examinations were made of normal sera or tissues, it would not be possible to detect presence or absence of a particular component which might be correlated with capacity or incapacity to yield a particular antibody. It may, of course, be said that at some future date biological tests will become more subtle than they are at present and that then the individuality of the defective animal will be recognisable by antigenic analysis as an individualistic feature of protein structure which does not respond to the stimulus of a particular antigen.

Yes; one cannot discard such suggestions as futile, but there is another line of speculation which seems to me better worth considering. This puzzling fact of individuality may be attributed to some condition in the general "make up" of the circulating plasma; and one must remember here that, when the transition takes place from the dynamic state of the living plasma to the relatively static condition of the serum, the latter condition is a very imperfect representation of the former. The individuality in question may reside in the former without transmitting any clue to its identity in the latter. To express this suggestion in its simplest form, the animal which is relatively unresponsive to an antigen may in reality have the material for producing the correct antibody and may actually form it, but the activities of its plasma may prevent it from settling down into a stable condition, with the result that it disappears from the serum.

It should also be realised that there is no cogent reason why the production of its exact counterpart should be the inevitable result of an antigenic stimulus. If, as on my view, the animal's idiosyncrasy is a humoral property, the endothelium which has adsorbed antigen may dissociate from the plasma not an exact stereo-chemical "mirror" of antigen but some other complex which may have a less specific or even a non-specific combining affinity for antigen, or an affinity which may even be unrecognisable as an antibody to the antigen introduced. And, again, on the secretory hypothesis of antibody formation, it is equally possible that the cellular stimulus may not be rigidly specific; something may be secreted which is not a precise antibody to the material introduced.

There is another possible complication. It is not necessary that the new antibody which appears in the circulation should remain unaltered in this medium. Fresh combinations may here arise with other constituents of the plasma and may modify the characters of the antibody originally formed. This is another reason for expecting irregularities in antibody production.

Hence one may come to the conclusion that the production of antibodies is often irregular because immunisation may involve changes which are "non-specific" in the sense that some of the new activities may not be specially selective for the antigen employed, thus breaking down the demarcation between specific and non-specific immunisation. The combining capacity of an antibody does not always justify the inference that the antigenic material

possessed a corresponding structure (*e.g.* a "group" antigen). Whilst there may be non-specific effects attributable to a single antigen, this is even more likely to be the case with the complex of antibodies produced by a complex of antigens. The different antigens do not always act individually and independently; and it would be unsafe to assume that the antibody output can always be used as a reliable mirror for analysis of the antigens.

There is also the contingency that irregularity of antibody production may be due to the condition of the antigen. Its specific combining affinity, *abc*, may be hampered in its activity by close proximity to *d*, *e*, etc.; then the antibody formed may differ from the mirror of *abc*. Thus a non-specific result (or failure) of immunisation is not sufficient to prove absence of an antigen; it may have been present in a masked condition.

THE ANTECEDENT CONDITION OF ACTIVE IMMUNITY.

When serological antibodies are obtained by immunisation, what was their antecedent condition in the circulation of the immunised animal? This question, which I have already raised with reference to animal idiosyncrasies, needs further attention, because confusion has often arisen from the tacit assumption that the characters of antibodies *in vivo* and *in vitro* are the same.

It is known that profound chemico-physical changes take place during the transition from the living plasma to the dead fluid which is obtained by bleeding; then, during the coagulation of the blood, there are further violent alternations which must leave their impress on the serum. So it is improbable and certainly cannot be taken for granted that the antecedents of serological antibodies are unaffected by these changes or, conversely that a serological antibody is identical with a property operative in active immunity. Evidence of this difference in the properties of antibodies is provided by two main types of experimental data.

(1) Active immunity may be acquired without the production of effective serological antibodies; *i.e.* the serum is not always antibacterial *in vitro* and its passive introduction does not always confer resistance on a susceptible animal. The thesis that such immunity is cellular and not humoral does not seem to me to be satisfactory. It is both; it is true that there must have been a cellular Umstimmung, but this is not sufficient to deal with the invasive bacteria; the second and equally essential factor is a change, consequent upon the Umstimmung, in the combining properties of the circulating plasma.

(2) In the course of active immunisation, when antibodies are known to have been formed, it is often found that there are striking differences between the behaviour of the circulating antibodies and the properties of those recoverable in the serum.

I discussed this subject in my paper on "The capillary endothelium"[1] with particular reference to Madsen's views on the formation of diphtheria antitoxin.

[1] *J. Hyg.* **22**, 355, 1924.

As a result of a preceding dose of toxin, a horse already contains antitoxin in its blood, as shown by titration. Then a second dose of toxin is given. *In vitro* this dose would be neutralised by a few c.c. of the horse's blood and would then produce no effect if inoculated into the horse; *in vivo* the toxin is not neutralised by the very much larger amount of circulating antitoxin but stimulates the production of more antitoxin. Again, if this same dose of toxin is inoculated into a passively immunised horse, which possesses therefore only a slight concentration of serological antitoxin, it is neutralised and produces no reaction.

It is curious that serological antitoxin possesses, both *in vitro* and in passive immunisation, a potency which is not forthcoming in the acquired, circulating antitoxin from which it was derived. One is reminded of the behaviour of the normal rabbit towards the anthrax bacillus; its circulating blood is not antibacterial but its serum is.

The above example will suffice to show that there are substantial experimental reasons for refusing to take it for granted that acquired, circulating antibodies are identical, in their behaviour, with the antibodies demonstrable in the serum. Then what is to be said about this antecedent condition?

In the first place, observed differences may be partly due to differences in environment. It is known that antigen-antibody reactions *in vitro* depend upon suitable chemico-physical conditions and will not take place unless this requirement is satisfied; similarly, in acquired active immunity a favourable medium is essential for the reaction. Thus it is quite possible that differences in the activity of antibody may be evident, although the actual property, termed antibody, is one and the same. For example, conditions may be favourable in the immunised animal but unfavourable in the serum (active immunity with no corresponding serological action); or the reverse may obtain (reaction between antigen and antibody *in vitro* but co-existence of the two, without neutralisation, in the immunised animal).

But it is sometimes clear that the above explanation is inapplicable. Why, for example, should serological antitoxin, introduced *in vivo*, behave differently from actively acquired, circulating antitoxin? Here the environment is the same in both cases. Again, the fluctuations in serological titre during immunisation, including the appearance of "positive" and "negative" phases, are strong evidences that the antibody itself, irrespective of its environment, is subject to change.

Merely quantitative change in content of circulating antibody does not seem a sufficient explanation. I refer in particular to Madsen's idea that during immunisation there is a continuous destruction of the antibody which is being formed, though this loss is more than counterbalanced, during the "positive" phases, by the excess of antibody produced. This hypothesis would not explain the observation, quoted above, that a second dose of toxin is not neutralised by the enormously greater amount of antitoxin known to be present in the circulation.

Here, I think, one is forced to the conception that an antibody is subject to qualitative change. The readiest explanation is that, in active immunity, antibodies are often in a highly labile condition. In the transition to serum they may disappear or they may settle down into a more stabilised form.

Again, there may be differences in the lability of their antecedent condition, varying with the stage of immunisation. The consequence will be a difference in the serological output, depending upon the amount of the antecedent form which is capable of becoming stabilised when the transition takes place. This idea of variation in capacity for stabilisation I propose to substitute for the hypothesis that antibodies in active immunity are always undergoing destruction. Undoubtedly there may be instability and varying degrees of efficacy *in vivo* and there may often be "destruction" in the transition from living plasma to serum; but I do not recognise any "law of nature" that, apart from the possibility of individual idiosyncrasies, the animal body should regularly "destroy" the antibodies which it has elaborated.

Some of the data observed in the production of diphtheria antitoxin may be interpreted as follows: (*a*) *Negative phase.* When a horse receives a second dose of toxin, it is adsorbed by the cells which turn out antitoxin and produces a temporary disturbance in the mechanism of this process, causing the antitoxin to assume a more unstable type; less of it is stabilised in the serum, though there need be no quantitative decrease in the circulation. (*b*) *Positive phase.* The disturbance subsides in a few days and then there is increase in the type of antitoxin which will assume a stabilised form in the serum. (*c*) *Subsequent decrease in titre.* The adsorbed toxin is broken up (though it leaves a cellular Umstimmung) and this change is followed by increased instability of the circulating antitoxin, not necessarily accompanied by a quantitative decrease. (*d*) *Titre raised by metallic salts.* The salt is adsorbed by the surface of the cells concerned in antibody production and causes the antibody which passes through to assume a form which will be more readily stabilised.

This conception of a qualitative variation in antibodies may now be brought into line with what I have said above about the production of specific properties by a readjustment of the plasma's combining affinities. In some cases, if the new antibody, as produced *in vivo*, has the specific combining centre, *abc*, with a particular stereo-chemical configuration, this centre may be sharply defined and may produce a definite anti-bacterial or antitoxic effect on union with bacterial antigen; but in the transition to serum the group *abc* may be broken up, or may lose its particular stereo-chemical attribute, and then the serum will not combine with antigen. In other cases, the living *abc* may be more or less closely associated with other side-chains, *d* or *e*, which hamper its action so that union with antigen is imperfect or easily dissociated; but in the transition to serum *abc* may be freed from this encumbrance, so that it will readily make sharp union with antigen.

Similarly with serological antibodies, the *abc* may or may not be hampered by *d* or *e* and may thus present differences in "avidity," which may be influenced by the environment provided for the reaction with antigen. Thus there may be a difference between the avidity observed *in vitro* and that which is found when the serum is used for passive immunisation.

Further, as regards the rise and fall of antibody output, one may imagine that the active *abc* on the endothelial surface is subject to continued "bombardment" by non-specific combining affinities in the plasma and that these eventually inactivate it, thus leading to diminution or cessation of the yield

of antibody. Again, after this stage has been reached, one may introduce a new and non-specific material which has a combining affinity for the group masking *abc* and removes it; then there will be renewed antibody output caused by a non-specific stimulus.

More elastic conceptions of "antibodies."

In the preceding sections it has already been found necessary to make some departure from the precise definition of an antibody. It is not always an exact counterpart of its antigen; and it is not safe to assume that a serological antibody is identical with an antecedent property in the circulation of the actively immunised animal. Still, the major part of the definition has been observed; the antibody has been treated as a property due to the stimulus of foreign protein acting as antigen. Now I come to a much wider divergence.

The word "antibodies" is often used to designate some property considered to resemble that of a fully accredited antibody, although it is found in an animal which has never received the stimulus of the corresponding antigen. Here the term really means no more than a particular combining affinity for foreign protein. And "antibodies" may also assume a chemico-physical rather than a definitely chemical aspect; a chemico-physical state of the plasma or serum which promotes antibacterial reactions is often called an "antibody."

Elastic usage of the word, whether one likes it or not, is firmly incorporated in the literature and must therefore be recognised. In order to avoid ambiguities, I propose to write "antibodies" with inverted commas whenever reference is made to a property not referable to an antigenic stimulus.

The significance of alexin.

Something must first be said about "complement," "alexin" or "opsonin" in so far as these terms are regarded as representing a labile principle or "antibody" which is a distinctive factor in immunity. The work on this subject is extremely extensive and a good deal of it is out of date; but it may still be utilised, and appreciated, as a basis for effecting some reconstruction in the presentation of immunological problems. Though no current word is altogether appropriate for present-day requirements, "alexin" (without commitment to either Buchner's or Bordet's usage of the term) is perhaps the best of the three as a general designation of the labile principle; it avoids the element of bias in "complement" (as a presupposed adjuvant to "immune body") and in "opsonin" (which lays too much stress on phagocytic activity as the foundation of immunity).

In dealing with alexin it has been usual to follow the historical method and to begin with the facts observed *in vitro*, its properties, the failure to isolate it, the evidence of its complexity, and the indications that its properties are due to a chemico-physical state of the serum rather than to some special chemical contents. Then came the question of its existence *in vivo*, which is now practically settled in the affirmative; and, after this inevitable

conclusion was reached, there has been a disposition to infer that the mechanism of immunity in the living animal follows the pattern demonstrable in the test-tube, viz. co-operation between "immune body" and alexin. This method of treatment has been natural and was the only one available, because nothing could be said about alexin *in vivo*, even by way of inference, until a large array of serological data had been accumulated.

Now the time has come when advantage may be taken of these experimental results and the order of discussion may profitably be reversed, beginning with the true alexin (*i.e.* the labile factor in humoral resistance) as it occurs *in vivo*, and attaching no more than subsidiary importance to the remnants of alexin which survive in the transition from circulating plasma to serum. This indeed is the logical sequence; if alexin is of major importance, as I agree, its existence *in vivo* is the primary fact, not an assumption dependent upon deductions from experimental observation.

The living plasma is not serum, nor is it a mixture of plasma and serum; therefore it cannot be taken for granted that alexin *in vivo*, though it is the antecedent of serological alexin, is identical with the latter. Without any disparagement of serological data, one cannot accept them *en bloc* as representing biological conditions actually present in the circulation.

The readiest example is the fact that the plasma's activities take place at the temperature of the animal body; they are not concerned with what would occur at 0° C., or at 55° C. or at other impossible temperatures. This feature of alexin *in vivo*, that it constantly retains its activity at 37° C. or thereabouts, does not apply to fresh serum, where it would rapidly deteriorate at this temperature. This aspect of alexin as a property depending upon the vitality of the plasma now requires supplementing by more detailed conceptions.

First, what are its chemical properties? Attempts to isolate the alexin of fresh serum as a definite chemical complex have failed and the present tendency is to discard the purely chemical hypothesis and to regard alexin as a chemico-physical state of the serum constituents which promotes certain reactions but is not a special chemical participant in any one reaction. This alternative view contains an important element of truth, but not the whole truth as regards alexin *in vivo*. Instead of abandoning the chemical conception one must consider the possibility of enlarging it. Though in natural resistance towards a particular bacterium it is usually impossible to ascribe bacterial destruction to any one chemical entity, one may imagine the bacterium as being bombarded by a rapid succession of chemical combining groups, A, B, C, etc. A may not be directly effective but it may prepare a vulnerable point for B, and so on; the integrity of the bacterium will depend on its capacity to resist every chemical weapon directed against it. In this "bombardment" alexin must participate and it may manifest itself as a chemical factor, not as a single entity but as the activity of an indefinite number of the combining affinities present in the plasma. Thus the search for a chemical alexin may be said to have failed not because alexin possesses no chemical attributes but

because they are too numerous and diversified to be identified as a single complex (such as an enzyme).

Turning now to the physical aspect, it may be urged that the main attribute of alexin is not chemical (possession of certain combining affinities) but chemico-physical (a condition of unstable equilibrium which is necessary to promote complex reactions). This is no doubt true for experiments *in vitro*; here it is the unstable equilibrium of the fresh serum which is the important factor rather than some particular and labile combining affinities contained therein. It is also true that a condition of unstable equilibrium is essential to the property of alexin in the living plasma. But this condition is a property of the plasma as a whole; one cannot take it for granted that it may be assigned exclusively to certain components or that one can distinguish from these certain other components as being in the relatively inert physical condition of inactivated serum. Thus, chemico-physically as well as chemically, it is not immediately clear how alexin *in vivo* can be distinguished from other humoral properties or "antibodies" of natural resistance.

Further consideration is obviously required before treating this conclusion as final; but I may first point out that the above conception of alexin, which is admittedly nebulous, at all events covers the main requirements. It emphasises the high importance of alexin as one aspect of the defensive mechanism; and this is the principal fact, about which there does not appear to be any dispute. Alexin, interpreted in this way, may still be regarded as the great natural factor in immunity; and it may still be taken to comprise the properties known under the more attractively teleological terms of "complement" or "opsonin," *e.g.* in promoting the action of an immune body passively introduced, by facilitating lytic action or susceptibility to phagocytosis.

But the objection will be raised that too much is ascribed to alexin and nothing is left for the co-operation with another sort of "antibody," generally termed "natural immune body." Here there emerges an important question which may be expressed in general terms. The humoral mechanism of natural resistance is obscure and is known to be highly complex; if it could be interpreted as a dual mechanism that would indeed be a simplification; but to what extent is this explanation likely to be valid?

The tacit acceptance of this idea of a dual mechanism (co-operation between alexin and a natural "immune body") dates back from the time when it was taken for granted that immunological analysis was concerned with definite chemical entities and that, though their isolation in a pure state was not accomplished, a useful separation of them could be effected by simple physical methods, such as the application of gentle heat. Hence the distinction between "immune bodies," which survived this treatment, and alexin, which was destroyed. But the conception of alexin as a special chemical substance is no longer tenable and therefore the evidence in support of the dual mechanism needs revision and one must review the new position with an open mind.

To illustrate my meaning, it may be assumed that a bacterium is modified by union with a circulating combining affinity, A, and is then reduced to a non-viable condition by further union with B. This may quite well happen *in vivo*. Moreover, if the circulating plasma is converted into serum, it is quite possible that both A and B may survive, and there is the further possibility that A may be found more stable than B. All this is accepted as a quite possible example of a dual mechanism working *in vivo*. But now one must raise difficulties. A and B need not necessarily survive in the serum. Or, if they do, they need not differ in stability; they might do their work equally well *in vivo* if they were both found, serologically, to be either stable or labile. And why imagine that out of the innumerable circulating combining affinities all are inert towards the bacterium except A and B? Not very likely; there might easily be a dozen or more with some influence on the bacterial surface. Where is the dual mechanism now, either according to the Ehrlich or the Bordet pattern?

In certain cases the co-operation of two distinctive factors is obvious; *in vivo*, when a true antibody is passively introduced and receives the assistance of circulating alexin; *in vitro*, when the antibody is activated by fresh guinea-pig serum. But these are artificial procedures; is there a similar co-operation in natural resistance?

A suggestive analogy may perhaps be afforded by certain experimental data which are free from the objection that the observation starts with a known mixture of different materials. In a fresh serum containing a known antibody it is sometimes possible to show that this antibody and alexin are separable.

Removal of the latter with retention of the former is, of course, easy; one is more interested in the converse part of the demonstration, removal of immune body with retention of alexin. This may be done by adsorption with antigen at 0° C., when immune body may be fixed and alexin left free, though this is not an invariable rule, as alexin is sometimes adsorbed at this temperature. It has also been shown that alexin may be retained by the pores of a filter whilst immune body passes through.

These experiments are of interest as showing that it may be feasible to effect an artificial separation of a given serum into two substances, the one carrying a relatively stable property and the other a property which is highly labile. They do not amount to proof that such separation actually exists in the active immune serum prior to treatment; and the deduction that the circulating plasma carries two such separate factors in natural resistance would be still more hazardous. At the same time they may be taken as suggesting that something in the nature of a dual mechanism may exist *in vivo*.

Is this suggestion supported by any more definite reasons for thinking that the combining affinities of the circulating plasma are of two sorts? Reverting to the idea of a "bombardment" on the bacteria, if there were always a master weapon, A, which made a direct hit and left a miscellaneous B, C, D, etc., to complete the damage, there would be a temptation to give A a distinctive title, such as "natural immune body," and thus to separate it from the other missiles which might be lumped together and be called "alexin." And this assumption might be taken to imply that alexin is endowed only

with the non-specific weapons and that it is this lack of specificity which distinguishes it from a more primary factor in an effective bombardment. But, with relatively few exceptions, there is no experimental basis for assuming that there is a distinctive A which can be regarded as specific. Natural resistance cannot be explained in terms of acquired immunity.

As regards differentiation by the application of gentle heat, it is not at all clear that this serological test indicates a sharp or radical distinction *in vivo* between two different classes of chemical combining affinities. The assumption that there may be such a distinction in natural resistance must, apparently, be based on an inference from what takes place when the stability test is applied to fresh serum. It may be thought that the combining affinities which survive represent antecedents in the circulation which are also relative stable and thus differ from alexin. This may be true to a certain extent. No doubt there are many varying degrees of stability amongst the circulating elements, but it seems quite arbitrary to sort them out in accordance with their response to a serological test at 55° C.

Another consideration emerges when one remembers the importance of alexin's physical properties. Its activity, as observed *in vitro*, is related primarily to a complex system of equilibrium and only in a secondary degree to the integrity of chemical units. Activity of a chemical substance must not be confused with the activity of a chemico-physical property which is an attribute of the medium as a whole. This distinction should be observed when quantitative terms are applied to alexin. Here *quantity* should mean *degree* of efficacy of a labile system, not *amount* of a special substance.

For instance, one finds in the literature the statement that alexin, unlike immune body, is not increased by immunisation; and this is regarded as an important difference between the two. The statement may be based on observations that the efficacy of alexin in a fresh immune serum does not differ from its efficacy in a fresh normal serum to which inactivated immune body is added; and then the inference is drawn that the same statement as to the unchanged efficacy of alexin is valid for conditions *in vivo*. Assuming for the moment that the inference is permissible, it is difficult to see that it is of any great chemico-physical significance. There is no particular reason to suppose either that the physical condition of the circulating plasma as a whole is likely to be altered, in this respect, by immunisation or that this condition is separable from the altered state produced by immunisation.

But the inference from *in vitro* to *in vivo* is decidedly not permissible. There is no evidence that the efficacy of alexin *in vitro* is an index of the efficacy of this labile principle *in vivo*. Animals of the same species may show marked differences in the potency of their serological alexin though it cannot be shown (*e.g.* in the guinea-pig, which is generally used for "complement") that there is a corresponding difference in their powers of natural resistance. The idea that degree of natural resistance depends upon "quantity" of alexin is an assumption devoid of proof.

In summary of this discussion I may say that, in my opinion, the mechanism of natural resistance is not "dual" but multiple (which includes "duality") and is not based on a replica of serological demonstrations of duality. This view, although it leaves alexin as an intangible complex of chemical and physical properties, seems to me preferable to the more orthodox attitude which first assumes that alexin *in vivo* is the same as *in vitro* and then destroys the illusion of greater precision by adding the necessary qualification that about the true nature of alexin very little is known.

In the following section I consider the other sort of natural "antibodies" which are supposed to co-operate with alexin.

Alexin and other natural "antibodies."

By the indirect method of serological analysis one can gain some insight into the intricacy of an animal's protective mechanism. Though the properties of the serum are not always a reliable index of the antecedent condition of the plasma, they reveal a high degree of complexity which indicates that there must be still greater, though qualitatively different complexity in the combining affinities of the circulating plasma. It can be shown that a normal serum may contain a large variety of "antibodies" which happen to fit particular antigens, although the latter had never been introduced into the animal's body.

Such idiosyncrasies of "antibody" emergence are not confined to the striking examples (*e.g.* of natural haemolysins) which seem to occur in haphazard fashion as peculiarities of an animal species. Further search for natural "antibodies" towards bacteria has recently attracted some interest. On obtaining the normal sera of various animal species and observing their reactions with a large variety of bacteria, natural "antibodies" of some degree of specificity towards one or another of these bacteria seem to have been found in considerable numbers. This discovery of a variable multiplicity of "antibodies" in normal sera enables one to form a more tangible idea, based on experiment, of the large range of natural combining affinities with which a bacterium may be "bombarded." For the purpose of exemplifying this point, further accumulation of similar data does not appear to be needed.

Experimentally, it is easy to show that many of such serological "antibodies" are sufficiently heat-resistant to be separable from alexin and that they can then be reactivated by fresh alexin. In this way an artificial dual mechanism is demonstrable. But there is no satisfactory evidence that there is a similar separation *in vivo* between the activities of combining affinities which are heat-resistant and of those which are not. All that can be said is that here is another aspect of natural resistance which shows that it is not entirely dependent on combining affinities which are labile. Nobody supposed that it was.

My conclusion is that careful consideration of these natural "antibodies" does not invalidate the opinion that, when dealing with vital activities, alexin

cannot be treated as a separable factor. The approach to natural resistance must be freed from any preconceived restriction that conformity with a dual mechanism is to be observed.

Combining affinities in natural resistance.

In considering natural immunity it is not desirable to think exclusively of distinctive examples where a bacterium is innocuous for certain animal species though pathogenic for others. One should take a wider view of natural conditions and include immunity towards saprophytes, natural resistance towards the feebly pathogenic bacteria which rarely gain a firm foothold, and so on, in a descending scale, until one comes to the slight and usually ineffective natural immunity towards highly virulent bacteria.

In all these examples one may say that the normal plasma constituents interfere, or tend to 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 that, when interference is successful, bacterial disintegration, often completed by phagocytosis, is the result. It cannot be assumed that this interference is always due to any one specially selective action on the part of a specific or non-specific "antibody" (*e.g.* a combining affinity, A, which is anticapsular). Such an agent is only demonstrable in special cases and, in its absence, one thinks rather of a systemic complex of combining affinities, an ordered succession of labile reactions between plasma and bacteria (or bacterial products), with the repeated occurrence of loose union followed by dissociation. This complex is effective in some cases but ineffective in others. How is the difference to be explained?

Reverting to the conception of a "bombardment" with A, B, C, etc., these weapons are not discharged in random fashion but are directed by that organisation of the plasma which constitutes the animal's individuality. Two animals may possess the same missiles (chemical affinities) but these may be discharged in different sequences (owing to differences in chemico-physical organisation). The sequence A, B, C, etc., may be effectively antibacterial, whereas B, A, C, etc., may fail. Here it is the sequence which makes the difference; neither A nor B nor C may be specifically anticapsular but the right sequence of intervention may prevent the bacterium from synthesising a protective capsule. Out of fifty reactions, for instance, each might be non-specific *per se*; but the possible ways in which these might be arranged in sequence are very numerous; the manifestation of animal specificity resides in the particular arrangement adopted. Here is one way of explaining the differences between natural immunity and susceptibility when there is no specific "antibody" to account for the former condition. Apart from its influence on bacteria, it is this kind of specificity which inhibits the growth of heterologous animal cells.

This significance of a chain of reactions involves the difference between the dynamic and the static conceptions of resistance, a difference which is directly concerned with the frequent lack of correlation between the resistive capacity of the living body and serological evidence of such capacity. In the change from the circulation to the relatively static condition of the serum, the animal's individuality is expressed in resultants, reactions which have

become stabilised. A specific sequence of events has been transformed into a specific arrangement of chemical groups, an arrangement which cannot be more than an imperfect representation of the activities upon which the animal's resistance depended. I have already raised this point in discussing animal idiosyncrasies in antibody formation. It is admittedly desirable, wherever possible, to replace vague biological ideas of "activities" by more precise chemical conceptions and one would like to be able to say that serological analysis of an animal's condition of resistance would, if sufficiently delicate, reveal the precise combining affinities upon which resistance depends. But here, as an explanation of natural resistance, one is asking for an impossibility. This difficulty is of constant occurrence in immunology; chemical precision proves inadequate and has to be supplemented by less tangible biological postulates.

Recognising this difficulty, one may say, with regard to the humoral factors in natural resistance, that there are some inferences as to their general nature which are in accord with clinical and experimental data. (1) They involve reactions which can only take place in the condition of unstable equilibrium which is provided by the circulating plasma; just as, on the minor experimental scale, certain immunological reactions *in vitro* require a medium which offers the unstable equilibrium of fresh serum. (2) On the chemical side, some of these reactions may be due to combining affinities in the plasma which differ in different hosts as regards structure or avidity; and, as these affinities are attached to protein carriers, their nature may partly depend on the character of the host's protein; there is always the possibility of such chemical individuality and, where it can be demonstrated, *e.g.* as a property comparable to a haemolysin, it is a valuable explanation. (3) But in the majority of cases the reactions cannot be explained as a single act of destruction exerted by a "master weapon" (like the germicidal action of a simple chemical); they must usually be ascribed to a sequence of events, where the first reaction prepares the way for the second, and so on. This sequence will depend on the individuality or systemic influences of the host's plasma, which determine an orderly sequence of changes with reversion to a particular equilibrium, just as in the life history of the tissues there seems to be a rhythmic cycle of change in the protoplasm of individual cells.

Relation of natural to acquired resistance.

In replacement of the "dual mechanism," I have endeavoured above to formulate some conception of the complex conditions in the living plasma which constitute a normal mechanism of resistance. This is the basis upon which acquired resistance is superimposed. The old mechanism is readjusted, as discussed in relation to the origin of serological antibodies, with emergence of new combining affinities and probably also with some rearrangement in the sequence of interactions between plasma and bacteria or bacterial products.

It is not simply the production of a new entity which co-operates with the normal factors as a "dual" mechanism.

The best example is the complete acquired immunity which ensues after recovery from some infections. Here there is a close resemblance to complete natural immunity; the new condition is not usually attributable to any special antibody but to the permanently acquired change in the general "make up" of the plasma, consequent upon the cellular Umstimmung. And also in less strongly marked instances of acquired immunity there is usually evidence, in greater or less degree, of some general or systemic change, not merely of the introduction of some new combining affinity which may be demonstrable as a serological antibody. Such antibody may be of the highest importance, *e.g.* in the prevention of capsule formation, but it would be unsafe to affirm that true immunisation against infection ever consists in nothing more than the production of a new combining affinity. The ideal of biochemistry (production of the precise counterpart to an antigen) is not to be identified with the ideal of therapeutics.

Now that I have stressed the importance of normal conditions as the basis of acquired immunity, I have some criticism to offer on the thesis that acquired immunity is to be regarded not as something new but rather as an enhancement of a natural mechanism. Such expressions as "enhancement" should be replaced by "reconstruction." It should be frankly recognised that the conditions of acquired immunity are definitely created *de novo*. There is not, I agree, a creation of new chemical entities out of material which was not present in the normal condition; and here I think my conception of readjusted combining affinities makes the change easier to understand. But there is undoubtedly an emergence in the plasma of properties which did not exist previously.

At this point it may be objected that I have not attached sufficient importance to natural "antibodies." I have discussed them briefly in a preceding section and have recognised that they are of interest; but I have not dealt with the question whether they are of major significance in the correlation of natural with acquired resistance. Upon this claim to their importance, opinions may be roughly divided into two opposite camps.

(1) From a sceptical aspect, one might say that in serological experiment an acquired antibody is usually a property which was definitely absent from the normal serum, not a property which was already present, though perhaps in a more rudimentary state. Animals which normally possessed some of the antibody would not be suitable for use in the production of antibodies required for precise antigenic analysis. And it does not seem at all likely that studies of the antibacterial action of normal serum can be made the basis of a general proposition that natural "antibodies" are the precursors of acquired antibodies. Acquired immunity, though admitted to be a development of pre-existing conditions, is not a development of pre-existing "antibodies" but is the acquirement of something which is definitely new. There is no satis-

factory evidence that the characters of a cell's normal secretions would throw any light on its ability to form specific antibodies in response to immunisation. The suggestion that it might is vague and difficult to visualise; it appears rather like a rehabilitation of the Ehrlich doctrine that antibodies are normally pre-existent, with the minor qualification that the pre-existent condition may be somewhat rudimentary.

(2) Those who think that the search for these natural "antibodies" has a bearing on the evolution of acquired antibodies may argue that the properties of the plasma are not independent entities but depend upon the nature of the cellular secretions which are turned out into it and these secretions are the real agents which determine the plasma's activities. The cells of the normal body are constantly producing substances which are likely to be more or less protective against bacterial invasion; specific immunisation is simply a specialised direction of this normal process. This view avoids the difficulties of the Ehrlich doctrine and places the essential elements of his theory on a sounder basis.

Of these two views I prefer (1) but admit that there is an element of truth in (2). The condition of the normal plasma must depend on cellular activities and it may be accepted as a general proposition that immunisation is a reconstruction of a normal condition. But I agree with (1) because I regard the change due to immunisation as taking place in the plasma; the cellular secretions, turned out into the plasma, are normal to begin with, but their combining affinities are changed when the plasma passes through endothelium which has been modified by adsorption of antigen. On (2), it is implied that the change occurs within secretory cells, the products of which have been modified by adsorption or ingestion of antigen. I have already given my reasons for preferring the former explanation of antibody formation. It accounts for the facts in a simpler way, without the postulate that there is an unlimited diversity, depending upon the nature of the antigenic stimulus, in a cell's secretory capacities.

Before leaving this question of the relation between natural and acquired resistance, I must point out an inevitable consequence which follows from the decision that the latter condition is, in some way, a readjustment of the former. It means that the latter, like the former, cannot be explained without the introduction of those vague biological conceptions which the precise chemist abhors. Then how far can the word *antibody* retain the original precision claimed for it in acquired immunity? Or, to put the question rather differently, what is the real significance of antibodies in acquired immunity? I think the answer to be gathered from what I have said in this article is: There are three aspects of acquired immunity with which the production of antibodies is concerned. (1) Antibodies may be formed which are the precise mirrors of their antigens. (2) By a similar but less precise mechanism, antibodies may arise which are not the exact counterpart of any particular antigen. (3) The antigenic stimulus may lead to complex humoral changes which are not identifiable as antibodies. The pure chemist will approve of (1),

will be very little interested in (2), and will positively dislike (3). The immunologist is equally concerned with all three aspects.

Discussion.

Current opinions about the nature of antibodies are well represented in the Medical Research Council's recent volume on Immunity[1]. Each of the contributors is a well known authority and I think that, in the following brief discussion, I can safely confine myself to articles contained in this publication.

All the authors are aware that they are dealing with an obscure subject.

Ledingham and Schütze (p. 102) say that "the virtue of antibodies in the processes of immunity has been much disputed and cannot be regarded as settled." Muir (p. 201) writes "...practically nothing is known with regard to the constitution of antibodies and the exact nature of their specific action." Browning (p. 207) states that "it is convenient to describe as antibody the specific alteration produced in the blood-plasma or serum of an animal which has reacted to an antigen, but it must be clearly understood that the term implies merely the acquisition or enhancement of specific properties, and that it does not correspond with a chemically defined substance." Dean's view (p. 425) is that "we have no direct evidence of any such substance as a precipitin, and we have no conclusive evidence that antibodies, in the sense of chemical substances, exist."

And here are three sentences from Browning's article on complement. (1) "As to the mode of action of complement there is no definite knowledge" (p. 347). (2) "The origin of complement is obscure" (p. 348). (3) "At present, it is impossible to determine whether complement is of a compound nature, as is believed to be the case for enzymes, or whether it may represent a physico-chemical state of a mixture of proteins, lipins and other constituents of the serum" (p. 350).

Perhaps the most urgent difficulty which has to be grappled with is the fact that immunology, in its theoretical aspects, is at present in a state of transition. The invaluable pioneer work was largely based on the assumption that immunological factors were definite chemical entities. This view, as is indicated in the above quotations, can no longer be accepted in its original form. But it still dominates current literature, which has become a curious sort of patchwork. The old ideas are reiterated again and again, but are interspersed with cautious safeguards to the effect that, after all, very little is known about the real nature of immunity. This attitude is not helpful unless it can be supplemented by efforts at reconstruction. What is needed is not rejection of well-established serological data but a rather different way of utilising them. They may be regarded as "stepping-stones" to the real objective, which is a better understanding of the vital processes involved in immunology; they are not necessarily the actual "building-stones" of true immunological structure.

For example, there are many puzzling facts about the production and behaviour of serological antibodies. These are problems which lead one to consider the antecedent condition in the circulation of the animal which

[1] *A System of Bacteriology*, **6**, 1931.

yielded the antibodies; and one is then forced to the conclusion that the behaviour of antibodies *in vivo* is not an exact replica of what is observed in the test-tube. Speculation as to the nature and mechanism of the antecedent condition is thus needed, though it must conform to the requirement that it should be compatible with the facts and should help to explain them. Similarly with the data about alexin. Knowledge of serological alexin gives some clues to what may be expected of alexin *in vivo*; but it does not justify the assumption that the latter is a separate substance like the fresh guinea-pig serum which is added to the specific reagents in a test-tube experiment. There must be reconstruction of ideas about alexin *in vivo*, with recognition that immunology cannot be built up out of purely serological data and that processes *in vivo* are more complex than the dual mechanism represented in the activation of an antibody by complement.

About the origin of antibodies there is considerable divergence of opinions.

Browning (p. 205) insists that the source of antibodies is the cells of the body and (p. 214) states that "of fundamental importance is the fact that antibodies appear as a sort of secretion in the blood-plasma." And Muir (p. 287) declares that "it is no longer a matter of dispute that antibodies are the products of cellular activity." Ledingham (p. 70) says that the question as to the site of antibody formation "is now intimately bound up with that of the functional activity of the reticulo-endothelial system." He also makes frequent references to "the capacity of endothelial cells to elaborate antibody," though he admits (p. 33) that "of the mechanism of antibody formation by the cell we know next to nothing." Browning's comment (p. 215) on the view that the reticulo-endothelial system is the site of antibody formation is that "as yet the evidence for this cannot be regarded as conclusive." Adopting quite a different attitude, Dean (p. 434) is opposed to a secretory or vitalistic theory and revives the old idea that antibody is formed in the plasma and is due to combination with an antigenic element, which it retains. "It seems possible," he says, "that the injected foreign protein may enter into combination or be mixed with the blood-proteins so as to form a complex precipitable in the presence of a further quantity of the same foreign protein. Such a conception implies the persistence of antigen in the blood."

In a preceding section I have endeavoured to steer an intermediate course. I accept a vitalistic cellular activity as participating in antibody formation, but I find it difficult to believe that antibodies are manufactured by cells as an internal secretion and are then turned out, ready made, into the circulation. I prefer a hypothesis of filtration, which I ascribe to a function of endothelium, though I do not support the thesis that antibodies are secretions of a "reticulo-endothelial system." Dean's view, as regards persistence of an antigenic element in the antibody, does not seem to me to be tenable, though I agree with his protest against the assumption that antibodies are cellular secretions, turned out into the blood as new and specific chemical substances. I think he is right in regarding antibody formation as involving a change which is produced directly in the plasma, though I do not consider this to be the complete explanation of the mechanism. The compromise which I offer is the hypothesis of a changed cellular activity (in endothelium) which produces a humoral change (modification of the plasma by filtration).

Another question involving the origin of antibodies is their relation to what are called normal "antibodies."

Ledingham (p. 37) quotes, apparently with approval, the view that "normal serum contains an amboceptor of relatively non-specific type capable of acting as a sensitiser in conjunction with complement." He also thinks (p. 39) that the recent search for normal antibacterial "antibodies" is important in view of "the light it might throw on the development and properties of antibodies produced in response to immunisation." Muir (p. 201) says that "in the development of acquired immunity we see mainly an intensification of actions and properties possessed by the body in its natural state; we cannot say that any new method of protection appears." And (p. 362) he states that "it is a general law that the substances and the properties of an immune serum are the representatives and further developments of those in a normal serum." Browning (p. 219) suggests that natural antibodies are "a more or less unspecialised prototype from which the highly specific antibodies evolve in the course of immunisation....This conception obviates the need for supposing a specific receptor to suit every antigen."

Whilst agreeing that acquired immunity is due to the reconstruction of a normal mechanism, which is the main point, I have given reasons for thinking that it is not based on the conversion of a normal "antibody" into a specific antibody.

It is natural to find divergent opinions whilst there is the present uncertainty about immunological principles. It is admitted that revision must come at some future date, but there is a feeling of doubt as to whether the time is yet ripe for it. Anything like a final achievement is certainly not within sight, but that is not sufficient reason to postpone efforts at reconstruction. At present, it seems to me, attention is devoted too exclusively upon serological data, which cannot explain immunity without the aid of hypotheses about what is going on in the living body. Such hypotheses deserve more consideration, though they are likely to need frequent correction before they can gain general acceptance as valid explanations of immunological facts.

Summary.

It is best to begin with antibodies which conform to their original definition as new serological properties attributable to an antigenic stimulus.

Their origin is still disputed. They are often regarded as cellular secretions due to adsorption or ingestion of antigen. For my part, I consider that they are formed in the plasma by filtration through capillary endothelium which has adsorbed antigen.

The precise specificity which characterises many antibodies is difficult to explain. I have suggested that it may be due to an interplay of reactions between the *dextro-* and *laevo*-rotatory forms of particular combining affinities.

But antibodies are not always the exact counterparts of their antigens. I have discussed some of the possible reasons why animal idiosyncrasies may be responsible for irregular results. A defective yield may also be due to the antigen, which may be present in a masked condition.

It is often taken for granted that the characters of the antibodies found in the serum of an actively immunised animal are identical with those actually present in the animal's circulation. I have given reasons for thinking that this assumption is frequently erroneous.

Coming now to the more elastic conceptions of "antibodies," which relate to properties not attributable to an antigenic stimulus, the part played by alexin in the circulation of the normal animal has first to be considered. It is not a distinctive chemical entity, nor is it simply a chemico-physical condition of the plasma; it is a complex of chemical and physical factors which defy analysis. The dual mechanism represented *in vitro* between alexin and "immune body" does not afford a true picture of alexin's activities *in vivo*.

The humoral factors in natural resistance are too complex to be regarded as a dual mechanism. The difference between susceptibility and resistance often depends on that precise sequence of events in the plasma's activities which is peculiar to the species or even to the individual.

Acquired immunity is a reconstruction of the normal mechanism, with the emergence of a definitely new property. It is not simply the introduction of a new antibody but involves also utilisation, with readjustment, of the complex biological conditions normally present. As a result of the antigenic stimulus: (1) antibodies may be formed which are the precise mirrors of their antigens; (2) by a similar but less precise mechanism, antibodies may arise which are not the exact counterpart of any particular antigen; (3) the antigenic stimulus may lead to complex humoral changes which are not identifiable as antibodies.

Current opinions on immunological principles, as expressed by well known authorities, exhibit divergencies which cannot be satisfactorily reconciled with each other. There is, however, general recognition that the present status of knowledge is highly deficient. Though the time is not yet ripe for anything like a finally satisfactory reconstruction of these principles, efforts in this direction are needed, with readjustment of the old ideas by the aid of hypotheses about what is going on in the living body.

(*MS. received for publication* 7. XII. 1932.—Ed.)