XLI. A GENERAL REVIEW OF THE RECENT WORK IN IMMUNITY.[1]

By PAUL EHRLICH.

Two years have elapsed since the appearance of my "Collected Studies in Immunity" in Germany, and now that the book is about to appear on the other side of the ocean it is a pleasure for me to review briefly the progress made in that time, naturally without pretending to give a complete résumé of the literature.

I may at once say, however, that very little really new has been added to the views formulated by myself and my collaborators, and that the stereochemical conception of the immunity reaction, despite numerous attacks, has proven itself able to dominate every phase of the subject.

The arithmetical view of the toxin-antitoxin reactions and their analogues, which was introduced chiefly by Arrhenius and Madsen, has invariably shown itself to be untenable. It has led to a numerical science which is far removed from the principles of biological investigations and from the experimental results underlying these. On the other hand, so able an authority as Nernst at once recognized that the laws of chemical equilibrium are not applicable to mixtures of toxin and antitoxin. In addition to this von Dungern, Morgenroth, and Sachs have collected considerable new experimental evidence which demonstrates absolutely that the toxin-antitoxin combination gradually becomes firm, although it may in some instances be quite loose in the first stage. The complex constitution of the poison solutions has thus been conclusively demonstrated; and I may also remind the reader that there can also no longer be any question as to the independent existence of toxons in diphtheria poison, for van Calcar has succeeded in a direct separation of these bodies.[2]

[1] This chapter is written expressly for this American edition.

[2] van Calcar effected this by means of an ingenious dialyzing procedure (Berlin. klin. Wochenschr. No. 39, 1904). Certain objections raised by Römer

In view of the extraordinary success which physical chemistry has scored, it is readily understood how tempting it was for so eminent a representative of this science as Arrhenius to apply its principles to the new field of immunity. I have always emphasized the chemical nature of the reaction, and am glad therefore that the attempt to apply these principles has been made. It has demonstrated anew that the phenomena of animate nature represent merely the resultants of infinitely complex and variable actions, and that they differ herein from the exact sciences, whose problems can be treated mathematically. The formulas devised by Arrhenius and Madsen for the reaction of toxins and antitoxins explain absolutely nothing. Even in particularly favorable cases they can merely represent certain experimental results in the form of interpolation formulas. Neither do I believe that the phenomena observed in toxins and antitoxins bear any relation to the processes of colloid chemistry. The attempt which has been made to interpret the immunity reaction from the standpoint of colloid chemistry, a subject itself more or less obscure, is based on purely external analogies. I see absolutely no advantage in such a method, and I have grave fears that it will result in checking further progress along this line. Structural chemistry, on the other hand, has not only served to explain all the phenomena in immunity studies, but has also proved a valuable guide in indicating the lines along which further progress might be made. The limitations of colloid chemistry have already manifested themselves, and enthusiastic advocates of this science have been compelled to assume the existence of specific atomic groupings in accordance with my views. I therefore see no reason for abandoning the views expressed in my receptor theory, a theory in complete accord with the principles of synthetic chemistry. My decision finds additional support in the fact that the studies in immunity are constantly bringing to light new observations best harmonized with the views of structural chemistry. Thus I may remind the reader that Morgenroth has recently very cleverly proved the postulate that the components of the neutral toxin-antitoxin combination can be restored. This author succeeded in completely recovering the two components of a neutral mixture of cobra venom

(Berl. klin. Wochenschr. No. 8, 1905) have been effectually answered by van Calcar by means of some additional experiments, and by the demonstration that the membranes employed by Römer were unsuitable (Berl. klin. Woch. No. 43, 1905).

and antitoxin by means of an ingenious method. But even here we are not dealing with a reversible reaction, for it requires certain manipulations to disrupt the neutral combination; thus, in the case of cobra venom, the addition of hydrochloric acid is necessary. The neutral cobra-venom-antitoxin combination therefore behaves like a glucoside, which in itself is entirely stable, but is split up by the addition of hydrochloric acid.

Besides this, the interesting investigations recently published by Obermayer and Pick,[1] on the production of immune precipitins by means of chemically altered albuminous bodies, are of particular significance in connection with the chemical conception of the immunity reaction. These authors succeeded, by iodizing, nitrifying, and diazotizing animal albuminous bodies, in so changing them that, when introduced into the organism of the same or of different species, they excited the production of precipitins which lacked specificity. These precipitins, however, were strictly specific for their respective iodized albumins, xanthoproteids, or diazo-albumins, no matter from what animal species the albumins were derived.

We see, therefore, that the introduction of a certain chemical group into the albumin molecule completely alters the latter's power to excite the production of antibodies. This certainly corresponds entirely to the view that the production of antibodies is dependent on the chemical constitution of the exciting agent, a view which finds expression in my receptor theory.

The heuristic value of the receptor idea, the idea which underlies my side-chain theory, can best be appreciated by studying the development of our knowledge concerning the cytotoxins of blood serum. As a prototype of these substances the hæmolysins occupy a prominent place in this volume. The view that the hæmolytic immune bodies are amboceptors has been proven to be correct in every case, thus conclusively showing that Bordet's sensitization theory is untenable. To begin, the observations of M. Neisser and Wechsberg, that the action of bactericidal sera depends not only on the absolute but on the relative concentration of amboceptor and complement, presented conditions which could not be harmonized with Bordet's views. On the other hand, they were readily explained in accordance with the side-chain theory by assuming that the complement was deflected by an excess of amboceptor. But even if this expla-

[1] Centralbl. f. Physiologie, Vol. XIX, No. 23.

nation is not the correct one, as Gay has recently stated, it would in no way affect the soundness of the amboceptor theory. The existence of amboceptors is confirmed by so many experimental considerations that it is no longer a postulate of the theory, but is practically the direct expression of observed phenomena. The term amboceptor, of course, is used merely to express the two-sided affinity, to the cell on the one hand and to the complement on the other. The affinity of the amboceptor to the cell was demonstrated by the combining experiments published by Morgenroth and myself; and the direct union of amboceptor and complement is confirmed by a host of decisive observations. Of these, it will suffice to mention the test-tube demonstration of complementoids which occupy the complementophile groups of the amboceptor. This demonstration has since been effected in other ways (Fuhrmann, Muir, Browning, and Gay), so that the existence of complementoids is no longer evidenced merely by the possibility of producing anticomplements by means of inactivated serum, but is demonstrated primarily by the unmistakable interference of the complementoids in hæmolytic test-tube experiments. It is not necessary that complementoids should always exert an inhibiting action on hæmolysis; for it is obvious that changes in affinity may occur in consequence of external influences, physical, chemical, or chronological in nature. I believe that changes in affinity, either positively or negatively, are of the highest importance in correctly understanding the course of immunity reactions, although I do not deny the influence of certain catalytic factors on these processes (von Behring, Morgenroth, Otto, and Sachs). However, no general rule can be laid down. Experiments are constantly bringing forth surprises, but by diligent empiricism it is usually possible to bring the many different observations into harmony with a single point of view.

The original assumption, that amboceptor and complement (at least in the case of hæmolysins) exist free side by side, and that the complement does not take part in the reaction until the amboceptor has been bound by the cell (owing to an increase in the affinity of the complementophile group),—this assumption has not proven tenable in every case. In addition to the case described in a previous chapter by Sachs and myself, we now know of a number of combinations, discovered by Sachs, in which the amboceptor alone does not unite with the receptor of red blood-cells, or does so to only a slight degree. By combining with the complement, the amboceptor

has the affinity of its cytophile group increased, so that now it is able to unite with the cells. Thus far, such observations have been made only on normal amboceptors; and this fact explains why the numerous attempts of various authors to separate normal hæmolysins, by means of absorption at low temperatures, have failed.[1] The amboceptors obtained by immunization, on the other hand, regularly possess a high affinity for the cell-receptor. This is easily understood if we consider their mode of origin, for we may perhaps see in this a selection of the groups with the highest affinity. Certainly in this case the exception proves the rule; for the mere fact, that in some instances the amboceptor does not unite with the cell until it has first combined with the complement, at once shows that we cannot be dealing with a sensitization. On the contrary, this shows that the amboceptor is an interbody in the strict sense of the word. These conditions have been most clearly brought out by the experiments of Preston Kyes on cobra venom. The researches of Flexner and Noguchi, as we all know, showed that cobra venom by itself is no hæmolysin, but plays the rôle of amboceptor in hæmolysis. The most important of the activators is the one discovered by Kyes, namely, lecithin. The relation between snake venom and lecithin is really the same as that between amboceptor and complement; but the former possess one great advantage for chemical analysis,—they are both stable substances, and thus contrast strongly with the highly susceptible substances found in blood serum. Hence what was impossible in the case of the latter could readily be effected with cobra venom. Kyes, it will be remembered, has demonstrated, *ad ocular*, the direct union of cobra amboceptor and lecithin complement, and has furthermore succeeded in isolating the resulting combination, the cobra-lecithid, in pure form.[2]

Thus, for the first time, the conclusion was reached chemically

[1] In this connection I should also like to mention the interesting atypical behavior discovered by Donath and Landsteiner in the amboceptor reaction. These authors observed hæmolytic autoamboceptors in the serum of a patient suffering from paroxysmal hæmoglubinaria. These autoamboceptors, however, only united with the bloods at low temperature.

[2] Kyes has recently continued his studies at my laboratory, and has demonstrated the important fact that in this formation of cobra-lecithid there is a true chemical synthesis. The course of this synthesis is such that a fatty acid radical is split off from the lecithin molecule, whereupon the residual combination, which corresponds to a monostearyllecithin, unites with the cobra ambo-

which, as a result of biological experiences, I had always looked forward to.

The correctness of the amboceptor theory formulated by Morgenroth and myself is confirmed by another important link in the chain of evidence. As far back as 1900, in the Croonian lecture, I stated that, according to the amboceptor theory, three antilytic antibodies were possible. In addition to the substances which act as anticomplements, we could conceive of antiamboceptors of two different kinds. One of these inhibits the action of the amboceptor by preventing the union of amboceptor and cell, the other by occupying the complementophile groups. So far as the confirmation of the amboceptor theory is concerned, it is evident that the demonstration of antiamboceptors directed against the complementophile group is by far the most important; for, owing to the mode of origin, the development of cytophile groups of the amboceptor as reaction products of the specific counter-group (the cell-receptor) is self-evident. It was therefore particularly gratifying when I found that Bordet had recently furnished the demonstration that the antiamboceptor developed with an immune, or with a normal serum, is usually directed against the complementophile group. This discovery very prettily demonstrates that the mechanism of hæmolysin action proceeds according to the amboceptor theory. The error contained in our earlier conception, that anti-immune bodies were usually antibodies directed against the cytophile group, is practically only an error in the localization of the point of attack. This must now be corrected by regarding the complementophile group as the point attacked by the antiamboceptor.

We know that it is possible to produce antiamboceptors by immunizing with normal serum, and Pfeiffer and Friedberger have shown that the action of the antiamboceptor serum extends to all the amboceptors of the animal species whose serum was used for immunization. These facts are only apparently a contradiction of the specificity of amboceptors, for the specificity of the amboceptors applies only to the cytophile group. On the other hand, we must assume that all the amboceptors of the same animal species are at least partly similar in structure so far as the complementophile

ceptor. This of course destroys the foundations of Noguchi's calculations, which are based on the assumption that the reaction is reversible; it also disposes of certain statements made by Bredig.

apparatus is concerned. In a way, therefore, the amboceptor bears the stamp of the animal species from which it is derived. In this connection I have already expressed my views in the article entitled "The Mechanism of the Amboceptor Action and its Teleological Significance" (Koch Festschrift, 1903): "In general, the specific amboceptors possess a uniform structure in their complementophile portions, whereas they differ to a high degree in their cytophile groups, whose physiological function is the absorption of foodstuffs."

The studies of antiamboceptors have demonstrated that this conception is correct. We see, therefore, that the specificity of the complementophile group of the amboceptor, a specificity based on the animal species, at once leads to a difference in the amboceptors obtained from different species by means of the same immunizing material. In our Sixth Communication on Hæmolysins, Morgenroth and I published certain experiments showing that by means of an antiamboceptor we had been able to demonstrate the diversity of the amboceptors produced in different animal species by injections of ox-blood. This statement still holds good, and its direct consequence demands that in the practical application of bactericidal sera, we should mix immune sera derived from different animals.

In view of Bordet's observation, however, we shall have to revise our interpretation in so far as the site of this differentiation is concerned; the difference is in the complementophile group instead of in the cytophile group. On the other hand, we must abandon the differentiation of partial amboceptors in one and the same serum by means of antiamboceptors, a differentiation which we proposed in the study on hæmolysins. It must not be thought, however, that the pluralistic conception of the amboceptor apparatus is thereby overthrown. This conception is supported by so many arguments of a different kind that the existence of partial amboceptors can be classed as one of the demonstrated facts in immunity. I may remind the reader that by means of mutual elective absorption it is possible to differentiate the strictly specific portion of an immune serum from the non-specific components which give rise to the group reactions. By this means the presence of different amboceptor fractions could be demonstrated in the same immune serum. The observations made by Morgenroth and myself on isolysins also speak strongly in favor of a multiplicity of amboceptors. In these the possible presence of antibodies acting on the complementophile portion of the amboceptor is absolutely excluded. Finally, if we glance at the con-

ditions existing among bacteria, we find the so-called group reactions showing that the receptor apparatus and the antisera possess a highly multiple constitution. This fact, as is well known, has here been of great practical value. We see, therefore, that the plurality of the amboceptors, so far as the cytophile group is concerned, is an assured fact; the differentiation by means of antiamboceptors directed against the cytophile group can therefore very well be foregone. The production of antiamboceptors against the cytophile group seems to encounter particular difficulties, for the complementophile group always finds the corresponding counter group in the organism more readily than does the cytophile group, and therefore is alone bound by the tissue receptors. It is possible that in order to successfully immunize with cytophile groups, it will be necessary to isolate these groups. The latter might be accomplished by neutralizing the complementophile group with the corresponding antibody, or by destroying this group (=cytophilic amboceptoids).

In any event these studies confirm the correctness of the amboceptor theory, i.e., that there is a direct combination of amboceptor and complement. To repeat, therefore, the specificity of the amboceptors applies:

(1) To the receptor employed in immunization, and this manifests itself in the configuration of the haptophore group; and

(2) To the animal species from which the amboceptor is derived. The latter kind of specificity shows itself in the structure of the complementophile apparatus, which, as we know, consists of a large number of individual complementophile groups. To this plurality of the complementophile groups there corresponds a plurality of complements as can hardly longer be questioned. So far as the constitution of the complement is concerned, the fact that it is made up of a haptophore and a toxophore group is sufficiently proven by test-tube experiments. The indirect method first employed for the demonstration of the haptophore group, namely, by the production of anticomplements, can therefore be dispensed with.

However, I am convinced that just as normal body-fluids so often contain anticomplements, it will also be found possible to produce these by immunization. But as Moreschi has well pointed out, the experiments by which it was sought to demonstrate the production of anticomplements are not absolutely conclusive. Recent studies by Gengou, Moreschi, and Gay have shown that in the immunization with serum, antibodies directed against the albuminous constituents

are formed which, by uniting with the corresponding albuminous bodies, possess the property of exerting anticomplementary effects. In this case, therefore, the anticomplement action is brought about by the interaction of two components, one present in the serum of the immunized animal and the other in the serum of that animal species whose serum was used for immunization (Moreschi). It is clear, of course, that here the dissolved albuminous substances, not the complements, were the antigens. This being the case, the demonstration of anticomplements produced by immunization becomes extremely difficult, and it must be left for future investigations to see whether it is at all possible to differentiate these substances from those antibodies against albuminous substances which exert an anticomplement action. So far as the mechanism of the described anticomplement action is concerned, I do not think that the observations of Moreschi and Gay, that absorption of complement is associated with precipitation, necessarily mean that precipitation and anticomplement have any causal relationship. In fact it seems reasonable to assume, in accordance with Gengou's first explanations, that the property of binding the complements is exercised by the albuminous bodies sensitized with the specific amboceptor. We would have to conceive this somewhat in this fashion, that just as when immunizing with cells, agglutinins and amboceptors are formed, so also when immunizing with dissolved albuminous bodies two kinds of antibodies are formed, precipitins and amboceptors. If the latter, however, are really amboceptors in the sense of Ehrlich and Morgenroth, we must demand that they will have the same properties which we have always ascribed to the amboceptor type. As a matter of fact, the experiment shows that this is the case. These albumin amboceptors also, in order to react with the complements, must have the affinity of their complementophile apparatus raised, only in the present case this is effected by the combination of the amboceptor with the susceptible body, the albumin. We see, therefore, that this anticomplementary action corresponds to the deflection of complement through an excess of immune body, first described by M. Neisser and Wechsberg. Only in this case the deflecting amboceptor is of a different kind, and needs first to react with the corresponding receptor.

Through the researches of Wassermann and Schütze and of Uhlenhuth, one class of antibodies against dissolved albumins, namely, the precipitins, has been used, as is well known to differentiate albuminous bodies of various origin. These have thus come to be successfully

employed in the forensic demonstration of the origin of blood-stains. The same thing, of course, was possible in the case of the albumin amboceptors.

This fact has recently been taken advantage of by M. Neisser and Sachs,[1] who have devised a procedure by which, by deflecting hæmolytic complements by means of albuminous bodies loaded with amboceptor, they diagnosticate human blood, etc. The study of immunity thus furnishes two biological methods for deciding a point of vital importance in forensic medicine, namely, the origin of blood-stains. Considering the extreme importance of tests of this kind, I am convinced that hereafter it will be well to use this method in addition to the well-tried Uhlenhuth-Wassermann reaction.

This brief résumé, I believe, covers the chief points which have recently come up for discussion, and it is indeed gratifying to me that all the vital questions have been decided in favor of my views. I have gladly applied the results obtained in experimental investigations to an extension of my views, for it is obvious, considering the rudimentary character of a new science, that any successful prosecution of the work will also extend the theoretical conceptions. If then, in spite of this, all the facts brought to light fit naturally into the views formulated by me, I regard this as additional evidence that these views are not so much a theory as a necessary abstraction of the observed facts, an abstraction which is necessary not only in order to obtain a clear and harmonious conception of all the various observations, but also to furnish a scientific basis for a further successful development of the subject.

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[1] Berlin. klin. Wochenschr. No. 44, 1905, and No. 3, 1906.