ANTIBODY STUDIES[1]

I. REVERSAL OF THE ANTIGEN-ANTIBODY REACTION

F. M. HUNTOON

From the Mulford Biological Laboratories

Received for publication October 29, 1920

INTRODUCTION

Since it was first recognized that certain substances, such as bacteria or their products or certain other poisonous substances of animal and vegetable origin, on their introduction into the animal body, stimulated the formation of neutralizing bodies, which were termed antibodies, there has been constant investigation of the laws governing such neutralizations. Nevertheless, in spite of the enormous amount of work that has been accomplished in this field, the true nature of neither antigen nor antibody has been definitely established.

The present series of papers is concerned with attempts to secure purified solutions of antibody, which would allow direct methods of examination as to their nature and which could be used therapeutically without the introduction of extraneous material, having a dangerous or disagreeable effect.

The purification of antibody, as it has been attempted, may be accomplished by the direct and indirect methods.

The direct methods involve precipitation of the antibody together with certain of the other serum constituents and the redissolving of such precipitates. Such precedures must necessarily fail to give pure solutions.

[1] Presented in abstract form November, 1919—Pathological Society of Philadelphia—Reported in Proceedings of Pathological Society of Philadelphia. 1920, Volume XL Old Series, Volume XXII New Series.

The indirect methods may be classified as:

1. Direct adsorption methods.
2. Indirect adsorption methods.
3. Absorption methods.

All such indirect methods involve the use of some formed material with which the antibody combines; after the combination has been washed free of most of the other serum constituents the antibody is then removed from its attachment.

Since the present work is concerned with the indirect method, only this phase will be reviewed.

THE USE OF INERT ADSORPTION MATERIALS

It has been shown by several observers, Andrejew (1), Landsteiner and Reich (2), Jaque and Kunz (3), that animal charcoal, kaolin and other substances take up antibody from serum solutions, together with certain of the other constituents, and that freeing of the antibody from this combination is excessively difficult in the test tube, but that this may occur in the animal body. Ssbolew (4), using iron hydroxide with well diluted immune serum, obtained a precipitate which contained practically all the antibody content. Emulsions of this precipitate, when injected into the animal body, acted as antibody to subsequent injections of antigen, demonstrating that dissociation of the combination had taken place with a freeing of the antibodies. However, all attempts to cause a similar dissociation in the test tube failed.

INDIRECT ADSORPTION

Gay (5) (6), Zinsser (7), Muir and Martin (8) found that when a serum containing the precipitin antibody was brought in contact with an appropriate precipitinogen, the resulting precipitate carried down with it a large proportion of all the antibodies contained in the serum.

Gay and Chickering (9) and Chickering (10), making use of this phenomenon, purified antipneumococcus serum; Weinstein (11) purified an antityphoid serum and obtained antibody

solutions by dissolving such precipitates in weak alkalis. Although such solutions contained antibody, they also contained a definite amount of the serum proteins, since the precipitate in such cases is, at least in part, derived from the serum.

ABSORPTION METHODS. REVERSIBILITY OF THE ANTIBODY-ANTIGEN REACTION

It was early recognized that antibody would combine with its homologous antigen and in the case of formed antigens such a combination could be washed free of most of the other serum constituents. Many attempts were made to reverse this reaction and so obtain antibody in a more or less free state.

Aside from the obtaining of serum-free antibody, the question of the true reversibility of the reaction deserves attention and this will be taken up at a later point.

Landsteiner (12) and Landsteiner and Jagic (13) washed red blood cells agglutinated by abrin in many changes of salt solution and then by treatment with salt solution at temperature of 42°C. to 45°C. they were able to demonstrate that a certain amount of the abrin was split off from the combination, and rendered reavailable. They also showed this to be true for normal hemagglutinins. Finally, they demonstrated that continued washing with salt solution removed some bacterial agglutinins from agglutinated antigen, but that more were removed when such combinations were heated to 55°C. Although they had shown that with the normal hemagglutinins, more were rendered free by heating at 45°C. than at higher temperatures, they offer no explanation of the use of 55°C. for the dissociation of the bacterial agglutinin-agglutinogen complex.

Morgenroth (14) noticed that when red blood cells, sensitized with hemolytic amboceptor, were brought in contact with unsensitized cells, some of the amboceptor was transferred to the fresh cells.

Bail and Tsuda (15) working with the cholera vibrio, injected sensitized organisms into the peritoneal cavity of guinea-pigs and after destruction of the vibrio had occurred (Pfeiffer reaction),

they reinjected the guinea-pigs with unsensitized cholera vibrios, which were in turn destroyed, but as a rule somewhat more slowly. By bleeding the guinea-pigs after such treatment, they were able to demonstrate the presence of antibody in the serum. By digestion of sensitized antigen in salt solution at 56°C., they were able to show that bactericidal antibody was reavailable but not agglutinins; a result that has been confirmed in part by our own work with dysentery antibodies.

Spaet (16), also working with cholera and cholera immune serum, digested sensitized antigen in salt solution at 42°C. and demonstrated the splitting off of bactericidal antibody. In this work, sensitization took place at 42°C. and dissociation at the same temperature. Spaet found that more dissociation occurred when inactivated serum was used for sensitization than when active serum was used, and that when the antibody solutions were reheated to 56°C., this temperature did not cause a reduction of the titer but 66°C. caused a marked reduction.

Hahn and Trommsdorf (17) treated agglutinated bacteria with N/100 sulphuric acid and regained active agglutinins.

Von Liebermann and Fenyvessy (18), employing rabbit's immune serum against pig's corpuscles, digested sensitized antigen with N/100 HCl in salt solution. These extracts were precipitated with alkali, the precipitate dissolved and purified with ether. The final solution contained both agglutinins and hemolysins but showed no albumin with the most delicate tests used.

Kosaki (19), using rabbit serum containing immune hemolytic amboceptor against sheep's red blood cells, has contributed an important paper to this subject. Finding that dissociation could be brought about in solutions of sugar containing no electrolyte, he attempted to work out the laws governing the dissociation—such as the influence of temperature and volume. The claims that he puts forth in his conclusions are not substantiated in all respects by the experimental evidence presented. His suggestion that electrolyte is necessary to the combination of antigen and antibody is a generalization that has not been proved.

Also his claim to have recovered five-sixths of the antibody

combined with the antigen, while shown in the curve tables presented, cannot be substantiated by an analysis of the experiments shown in detail. On the basis of this claim, Kosaki thinks that all such combined antibody can be recovered; but such an assumption is open to doubt.

In all of the experiments of this nature, which are shown in the literature in sufficient detail to be carefully analysed, the following fact is apparent:

An excess of sensitizing serum has been always employed.

Since it is well known that an antigen will combine with not only sufficient antibody to cause a complete reaction (such as agglutination and hemolysis) but with many times this amount, and since, in no instance has it been shown that all the combined antibody has been recovered, it follows that a true reversal of the essential antibody-antigen reaction has not been demonstrated.

It is entirely conceivable in this connection that two distinct reactions are brought in play; one, the essential reaction, taking place between the antigen and the minimal amount of antibody necessary for complete sensitization and resulting presumably in a firm combination; the other, taking place between the antigen and an additional portion of antibody, a portion that is in excess of the minimal sensitizing quantity. The combination resulting from this reaction may or may not be a firm one.

The efforts to determine whether a complete reversal of antigen-antibody reaction is possible have been made with the use of minimal sensitizing quantities and also with greater quantities of the antibodies.

Considerable technical difficulties are presented in the use of a single sensitizing dose. We may state here, however, that several attempts, which we have made, both with hemolytic amboceptor-red cell combinations and with agglutinin-agglutinogen combinations have all failed to demonstrate any dissociation.

The work of others on dissociation of antibody in excess from its combination with antigen and, as will be seen in the following section, our own work show that only a certain proportion of attached antibody can be removed and that the amount remaining attached after such dissociation is, in all cases, somewhat

more than the minimal sensitizing dose. Thus, the assumption seems less reasonable that the antibodies are attached to antigen in two distinct portions in sharply different degrees of firmness than that a graduated variability in the firmness of the union exists, and that those antibodies which are less firmly bound are the first to be dissociated.

Although complete reversal of the antigen-antibody reaction may be impossible, there is abundant proof of the possibility of an incomplete or partial reversal enabling the production of antibody solutions in a more or less pure state.

The problem that we have undertaken was approached through the following subdivisions:

1. Improvements in the technic of obtaining antibody solutions free from other serum constituents.
2. Determination of the laws governing the dissociation of antibody-antigen combinations.
3. Study of differences in the dissociability of various antibodies from sensitized antigen.
4. Study of the chemical nature of antibody.

These question are taken up experimentally in the following sections.