precipitate formed by an antibody and its precipitin, as well as in that formed by an antibody and the precipitin to normal globulin. In agreement with this, Smith and Marrack[40] found that a precipitate formed by a precipitin and

[40] F. C. Smith and J. Marrack, Brit. J. Exp. Path. 11, 494 (1930).

a serum containing diphtheria antitoxin has the power of combining with diphtheria toxin; and similar results have also been obtained recently by Heidelberger and Treffers[41] in the case of specific predipitates formed by pneumococcus antibody with homologous antiserum.

[41] Personal communication of unpublished material by Professor M. Heidelberger and Dr. H. P. Treffers.

e. Factors Affecting the Rate of Antibody Production and the Specificity of Antibodies.—In order that an antibody be effective, the surface region of the antigen covered by an antibody end must be large enough so that the integrated attractive forces constitute an antibody-antigen bond of significant strength. With a bond of average strength the antibody molecule will be dissociated from its antigen template, perhaps with the aid of the auxiliary mechanism mentioned in Section VI, until an equilibrium or steady-state concentration is built up in the serum. Now if the antigen surface contains a large number of strong groups, capable of interacting strongly with complementary structures in the antibody, the antibody-antigen bond may be so strong that the antibody is not able to separate itself from the antigen, and only a small antibody concentration can be built up in the serum. We hence conclude that an antigen containing weak groups will in general be a good antigen, whereas one containing many strong groups will be a poor antigen, with respect to antibody production.

This prediction, which at first thought seems paradoxical, is in fact borne out by experiment. Thus a bland protein such as egg albumin is a good antigen, as are also conjugated proteins with weak groups attached. An azoprotein with many strong groups attached (arsenic acid, sulfonic acid, nitro, etc; the azo group itself is a rather strong group, capable of forming hydrogen bonds) is

a poor antigen; in order to obtain serum to such haptens an azoprotein containing a limited number of the groups must be used. I am told by Dr. Landsteiner that this observation was made in the early days of the study of azoproteins[42].

[42] See K. Landsteiner and H. Lampl, loc.cit.

Pertinent data have been reported in recent years by Haurowitz and his collaborators (loc.cit.), who found the optimum arsenic content for the production of antihapten by azoprotein made from arsanilic acid to be between 0.5 and 1.0%; very little antibody is produced by antigens with over 2% arsenic, although strong precipitin reaction is shown by azoprotein with an arsenic content as great as 10%.

A second deduction, relating to specificity, can also be made. To achieve a sufficiently strong antibody-antigen bond with an antigen containing only weak groups a large surface region of the antigen must come into play, whereas with an antigen containing strong groups only a small region (in the limit one group) is needed. Hence antibodies to antigens containing strong groups show low specificity, and those to antigens containing weak groups show high specificity. This prediction is substantiated by many observations. Egg albumin, hemoglobin, and similar proteins give highly specific sera, whereas azoproteins produce sera which are less specific, strong cross-reactions being observed among various proteins with the same hapten attached. This shows, indeed, that a single hapten group gives a sufficiently strong bond to hold antibody and antigen together. In such a case the approximation of the antibody to a strong hapten is very close, and great specificity is shown with regard to the hapten itself, this specificity being the greater the stronger the hapten. Many examples of these effects are to be found in Landsteiner's work.

f. The Effect of Denaturing Agents.—We made the fundamental postulate that the end parts of the polypeptide chains of the globulin molecule are characterized by having a very large number of accessible configurations with nearly the same

energy, whereas there is only one stable configuration for the central part. It is accordingly probable that the end configurations, giving characteristic properties to the antibodies, would be destroyed before the central part of the molecule is affected and, moreover, that the sensitivity to denaturing agents or conditions of antibodies to different antigens would be different. The available meager experimental information seems to be compatible with these idea[43].

[43] See Marrack, loc.cit., pp. 48-53.

Some remarks may be made regarding the difference in behavior of antibodies and antigens in the presence of denaturing agents. An antigen molecule may undergo a considerable change in configuration without losing completely its power of reacting with the homologous serum; if some of the surface regions remain essentially unchanged after partial denaturation of the protein, the antibody molecules complementary to these regions will retain the power of combining with them, whereas the antibody molecules complementary to the regions which have been greatly changed by denaturation will no longer be effective. In particular some native proteins may be built of superimposed layers, as described in Section IV. The antigenic regions on top of the top layer and on the bottom of the bottom layer would still be effective after the partial denaturation of the molecule by the unleafing of the layers, whereas the antigenic regions at the sides of the original molecule would in large part lose their effectiveness by this unleafing. The observation by Rothen and Landsteiner[44] that egg albumin spread into surface films 10 Å thick retains

[44] A. Rothen and K. Landsteiner, Science 90, 65 (1939).

the ability to combine with anti-egg-albumin rabbit serum is most simply explained by the assumptions that the native egg albumin molecule has the layer structure suggested above and that the process of surface denaturation of this

molecule involves the unleafing of the layers without the loss of their structure.

As mentioned above, it is probable that for most antibodies the end regions are affected by denaturing agents more easily than the central region, and that the first step in denaturation of an antibody involves these end regions and leads to loss of their specific properties. It has been shown by Danielli, Danielli, and Marrack[45] that the reactivity of antibodies is destroyed by surface denaturation[46].

[45] J. F. Danielli, M. Danielli, and J. R. Marrack, British J. Exp. Path. 19, 393 (1938).

[46] Rothen and Landsteiner (loc.cit.) have pointed out that from these facts regarding surface denaturation the conclusion can be drawn that "the specific reactivity of antibodies is to a large extent dependent upon structures different from those which mainly determine the specificity of antigens".

An interesting possible method of producing antibodies from serum or globulin solution outside of the animal is suggested by the theory. The globulin would be treated with a denaturing agent or condition sufficiently strong to cause the chain ends to uncoil; after which this agent or condition would be removed slowly while antigen or hapten is present in the solution in considerable concentration. The chain ends would then coil up to assume the configurations stable under these conditions, which would be configurations complementary to those of the antigen or hapten.

Many of the experiments suggested above are being undertaken in our Laboratories, with the collaboration of Dr. Dar Campbell.

VI. Processes Auxiliary to Antibody Formation

It seems not unlikely that certain processes auxiliary to antibody formation occur. The reported increase in globulin (aside from the antibody fraction) after immunization suggests the operation of a mechanism whereby the presence of antigen molecules accelerates the synthesis of the globulin polypeptide chains. There is little basis for suggesting possible mechanisms for this process at present.

The occurrence of the anamnestic reaction—the renewed production of antibodies to an antigen caused by injection of a second antigen—may be explained by the assumption that following the synthesis of an antibody a mechanism comes into operation in the cell to facilitate the removal of the antibody from the antigen, perhaps by changing the hydrogen-ion or salt concentration or dielectric constant. This would assist in removing antibodies not only from the second antigen but also from those molecules of the first antigen which had remained, covered with homologous antibody attached too firmly for spontaneous removal, in the cell. The evidence indicates that the anamnestic reaction is not in general strong. At a time after inoculation with typhoid bacillus or erythrocytes long enough that the corresponding agglutinins are no longer detectable in the serum injection of another antigen gives rise to the presence of these agglutinins in amounts detectable by the very sensitive agglutination test; but Kabat and Heidelberger[47] found that the amount of additional antibody

[47] E. A. Kabat and M. Heidelberger, J. Exp. Med. 66, 229 (1937).

to serum albumin produced by injection of egg albumin or typhoid toxin was too small to be detected by their method of analysis.

antigen?

The mechanism for catching the antibody molecule and holding it in the region of globulin synthesis may be closely related to that of antibody production—possibly a partially liberated globulin chain which forms a bond or two bonds with an antigen molecule directly above it is prevented from freeing its central part from the cell wall, and so serves as an anchor.

The renewed production of antibody in the serum after bleeding is to be attributed to the presence of trapped antigen molecules in the cells. The greater duration of active than of passive immunization may be attributed to this or to the presence of complexes of antigen and surrounding antibodies, the outer ends of which could combine with additional antigen.

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My interest in immunology was awakened by conversations with Dr. Karl Landsteiner; I am glad to express my gratitude to him, and to acknowledge my indebtedness to him for ideas as well as for facts. I wish also to thank Professors Michael Heidelberger and Dan Campbell for advice and assistance.

Summary

It is assumed that antibodies differ from normal serum globulin only in the way in which the two end parts of the globulin polypeptide chain are coiled, these parts, as a result of their amino-acid composition and order, having accessible a very great many configurations with nearly the same stability; under the influence of an antigen molecule they assume configurations complementary to surface regions of the antigen, thus forming two active ends. After the freeing of one end and the liberation of the central part of the chain this part of the chain folds up to form the central part of the antibody molecule, with two oppositely-directed ends able to attach themselves to two antigen molecules.

Among the points of comparison of the theory and experiment are the following: the heterogeneity of sera, the bivalence of antibodies and multivalence of antigens, the framework structure and molecular ratio of antibody-antigen precipitates, the use of a single antigen molecule as template for an antibody molecule, criteria for antigenic activity, the behavior of antigens containing two different haptens, the antigenic activity of antibodies, factors affecting the rate of antibody production and the specificity of antibodies, and the effect of denaturing agents. It is shown that most of the reported experimental results are compatible with the theory. Some new experiments suggested by the theory are mentioned.

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Legends for figures

Fig. 1. Diagrams representing four stages in the process of formation of a molecule of normal serum globulin (left side of figure) and six stages in the process of formation of an antibody molecule as the result of interaction of the globulin polypeptide chain with an antigen molecule. There is also shown (lower right) an antigen molecule surrounded by attached antibody molecules or parts of molecules and thus inhibited from further antibody formation.

Fig. 2. (A) Diagram representing agglutinated cells. (B) Diagram of the region of contact of two cells, showing the postulated structure and mode of action of agglutinin molecules.

Fig. 3. A portion of an ideal antibody-antigen framework. One plane of the structure corresponding to the value twelve for the valence of the antigen molecules is shown.

Fig. 4. A portion of an antibody-antigen network formed in the region of antibody excess.

Fig. 5. A portion of the network formed in the region of antigen excess.

Fig. 6. Representative soluble complexes formed with excess antigen.

Fig. 7. A soluble complex formed with excess antibody.

Fig. 8. The folding of polypeptide chains into a layer held together by imino-carbonyl hydrogen bonds.