THE MECHANISM OF ANTIBODY-ANTIGEN REACTION*

by

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INTRODUCTION

The reaction between antibody and antigen has been known to possess certain peculiarities not shared by other chemical or physical systems. These peculiarities became more evident with the introduction of quantitative chemical measurements of the component parts of the antibody-antigen reaction[1-3]. Numerous mathematical expressions have therefore been proposed in an attempt to apply the mass law to this reaction[4-7]. Some reviews of the theories proposed, and their historical perspective afford stimulating reading[8-11].

With three times crystallized yeast alcohol dehydrogenase[12] as a marked antigen, we were able to study the mechanism of antibody-antigen reaction using rabbit immune serum as the source of antibody. The results obtained and reported herein, suggested a new concept that places this reaction in better harmony with current advances in protein chemistry. A preliminary account of this work, in which enzyme inhibition and immunochemical methods were used, has already been presented[13-15].

The concept holds that following the first entry of an antigen into the body, antibodies are formed that are directed against one or more sites on its surface. When these antibodies react with antigen an antibody-antigen complex is formed. This complex (complex-I) can henceforth behave as a new antigen. The antibody that is formed to complex-I can, by an analogous process, react with the latter to form another complex antigen, complex-II. Complex III and IV, *etc.* are similarly formed. The extent of this complexing process is determined by the length and interval of the natural exposure of the subject to the antigen and, under experimental conditions, by the number of injections performed.

The implications of the above concept are as follows. (a) The antigen moiety of the complex can be sufficiently modified by its union with antibody to undergo an alteration of some or all of its reactive groups and become a modified antigen. The antibody formed against such altered surfaces may cross-react to a variable degree with the original unmodified sites on the free uncomplexed antigen. Furthermore, this modification may expose new antigenic groups that were heretofore structurally

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submerged and therefore immunologically inert. Antibodies to this new site would be highly specific and non-cross reacting. Alternatively, (b) the antibody moiety of the complex may be similarly modified by the antigen so as to render it strictly antigenic, or (c) an antigenic site may conceivably form at the junction of the antibody and antigen molecules.

The concept of the mutual modification of antibody and antigen resulting from their union finds support in numerous examples pointing to such alterations occurring in a protein in response to the same forces (van der Waals and ionic attraction, hydrogen bonding *etc.*) involved in antibody antigen interaction.

The alteration of the antigen may give rise to (a) antibodies that react with the altered and unaltered antigen or (b) antibodies that react only with the altered antigen. There are many examples of compounds, termed adjuvants, that presumably act on some substances to influence their immunological reactivity[9]. Some of these adjuvants are inorganic adsorbing agents such as alum, kaolin, magnesium compounds, *etc.*, others are biological products such as waxes, tubercle bacilli and typhoid vaccine. Of particular significance is the demonstration by LANDSTEINER AND SIMMS[16] that while purified Forssman's antigens produce no antibody response when injected into animals, they can be rendered antigenic by interaction with serum. Because such antigens remain physically intact it would be difficult to attribute the effect of the adjuvant to a process other than one resulting in structural alterations of the antigen incident to a physicochemical association with the adjuvant. The antibodies produced under these circumstances react with the native, unmodified antigen *i.e.* in the absence of adjuvant. Furthermore, culture filtrates of a strain of *Staphylococcus* were shown to affect nonantigenic homologous homogenates of rabbit muscle[17] and rabbit kidney[18] and autolysis to affect monkey brain[19] in such a manner as to endow them with a high degree of antigenicity when injected into the homologous host. It is reasonable to expect that the acquired antigenicity of these tissues was brought about through physicochemical alterations. The resulting antibodies in these instances also react with the native, unaltered antigen. There are still more striking and direct examples of structural alterations of a protein that give rise to the property of antigenicity. Most pertinent is the demonstration that the antigenicity of serum acquires new specificity by heat denaturation; such altered serum behaves like a new protein when injected into the rabbit. The antibodies so produced are highly specific and by contrast with the above do not react with the native, unheated serum[20–21].

The alteration of the antibody as a result of its union with antigen has been clearly shown[22] by the finding that when it reacts with an antigen there is a large increase in the molecular volume of the antibody. The type and magnitude of the volume change corresponds to that occurring in protein denaturation as was found following thermal denaturation of staphylococcus antitoxin[23]. In essence therefore, an antibody, by its union with the specific antigen, undergoes changes similar to those it manifests during heat denaturation. It is pertinent to reemphasize in this connection that serum acquires specificity by heat denaturation. It is not surprising, therefore, to find that a homologous antibody can become antigenic through an alteration involving increase in molecular volume, resulting not from thermal denaturation but from union with antigen. The modification of antibody by the antigen finds further support in the repeated demonstration that the antigenicity of horse antiserum is greatly increased by combination with antigen[24–26].

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A further projection of the above arguments into another aspect of immunochemistry suggests that the insolubility of specific precipitates of antibody-antigen complexes can be explained by the same basic principle. The resulting molecular expansion, akin to that occurring in heat denaturation, can result in exposure of hydrophobic groups to render the complex insoluble as it does in protein denaturation[27]. By analogy therefore, an antibody and an antigen can interact to produce a complex in which the antigen segment is sufficiently different antigenically to give rise to specific antibodies. The antibody portion may in turn be modified to an extent such that homologous antibodies are formed in response to this modified molecule which in its original, native state is not antigenic. In point of fact the antigen used in this work was indeed modified by its union with the antibody. This became apparent while studying the effect of antibody on the enzymic activity of the antigen. In aqueous media the free enzyme is ordinarily inactivated in a few minutes at 4°C. However, when combined with antibody its enzymic activity, though diminished, becomes very stable and is maintained for weeks under identical conditions.

The concept presented above can be expressed in simple terms as follows:

$$B + G \rightleftharpoons (BG) = C_1 \quad (1)$$

$$B_1 + C_1 \rightleftharpoons (B_1G_1) = C_2 \quad (2)$$

$$B_2 + C_2 \rightleftharpoons (B_2G_2) = C_3 \quad (3)$$

where G is the original antigen, B, is the original antibody formed against G. C_1, C_2, *etc.* are the successive complexes formed, B_1, B_2, *etc.* are the antibodies formed against these complexes respectively. Since it is difficult to isolate a single complex free from those complexes immediately preceding or following it, the term complex as applied to the experimental work denotes a range or a family of closely related complexes.

MATERIALS AND METHODS

Rabbits were the only animals used for immunization. All injections were given subcutaneously at weekly intervals and blood was withdrawn 7 days after the last injection. Each injection of antigen contained 10 mg of protein. The two antigens used were crystalline egg albumin and yeast alcohol dehydrogenase. Egg albumin was obtained commercially*. Alcohol dehydrogenase was prepared from baker's yeast by fractionation with acetone and ammonium sulfate[12]. It was crystallized three successive times with ammonium sulfate and stored in the frozen state in the salt at 0.5 saturation. Before injection into the animal the enzyme was dialyzed for two hours against running tap water at 6–8° C. It was found that with prolonged dialysis the enzyme lost considerable activity and became a poor antigen as judged by antibody response in rabbits. For the study of antibody-antigen interactions and for enzymic activity, the enzyme was not dialyzed. The desired amount was dissolved in 0.1% gelatin and kept on ice for a period not longer than ten minutes before use. Specific precipitates (complexes) were prepared with quantities of antiserum and antigen corresponding to those obtained at or around the equivalence point. At this point the molar ratio of antibody to antigen was 4:1 in the case of alcohol dehydrogenase and sera of rabbits injected 5 times. Specific precipitates were washed three times with 3–5 ml of saline at 0° C, suspended in saline and kept at 0–6° C until used. Quantitative micro protein analyses[28] were made on aliquots of specific precipitates dissolved in minimal amounts of 0.5 N NaOH. All turbidity measurements were made in the Beckman spectrophotometer at 340 mμ. In all instances where antibody-antigen interactions were studied, whether by turbidity measurements or protein analysis, normal rabbit serum was used as control and subjected to the same conditions of temperature, ionic strength, pH, and the various additions of antigen and antibody-antigen complex as the immune sera under investigation.

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EXPERIMENTAL

Since most of these studies were made with yeast alcohol dehydrogenase, it was necessary to investigate its properties as antigen. This was desirable inasmuch as our finding might well be related to possible peculiarities of this antigen. An investigation with the usual immunochemical methods revealed that the enzyme possessed most of the properties common to other antigens. When antiserum was treated with increasing amount of antigen unprecipitated compared to that precipitated was twenty fold or The amount of precipitate became maximal in the region of slight antigen excess, only to diminish markedly in the region of extreme antigen excess *i.e.*, when the amount of antigen unprecipitated compared tot hat precipitated was twenty fold or more (Fig. 1).

A study of the effect of antiserum on the enzymic activity showed that a considerable reduction of activity resulted from the combination of antibody with the antigen (enzyme). The resulting specific precipitates nevertheless showed definite enzymic activity in much the same manner as did specific precipitates obtained with crystalline yeast 3-phosphoglyceraldehyde dehydrogenase[29]. Evidently, the active surface of the enzyme was not covered by the antibody and remained available to the substrate. The mere precipitation of the enzyme cannot account fully for the inhibition of enzymic activity, inasmuch as the further addition of specific antibody

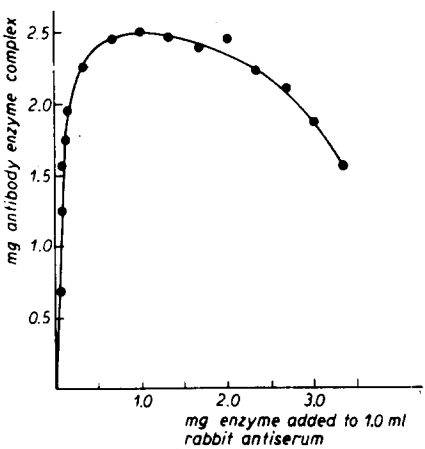

Fig. 1. Precipitin reaction with yeast alcohol dehydrogenase and its antiserum. The precipitin reaction consisted of 1.0 ml of rabbit antiserum and 1.0 ml of saline in each of several centrifuge tubes. Various amounts of alcohol dehydrogenase (antigen) were then added. The tubes were incubated at 0° C for 24 hours for maximum precipitation. The precipitate was then separated and assayed for protein (see text). The antiserum was obtained from a rabbit immunized with six injections.

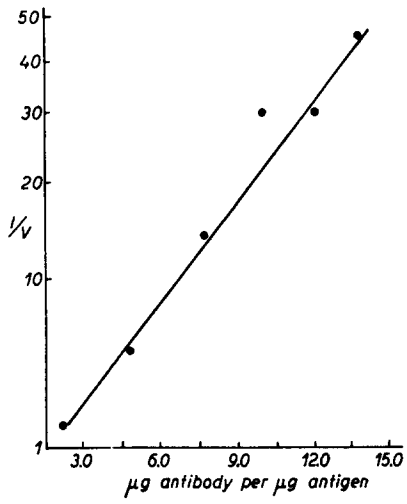

Fig. 2. Alcohol dehydrogenase activity of antigen-antibody complex as a function of antibody content. Antibody-antigen complexes were prepared as in Fig. 1. Aliquots of each precipitate were chosen to contain 32 γ of alcohol dehydrogenase and the enzymic activity was measured in the Beckman spectrophotometer at 340 mμ. The amount of enzyme in the specific precipitate was calculated from the amount added and that remaining in the supernatant fluid. The quantity of antibody precipitated was then calculated from the total protein precipitated less the enzyme. Initial velocity is expressed as the amount of reduced DPN formed per minute, one gamma of free enzyme giving a standard rate of 5×10^{-3} μmoles of reduced DPN.

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to the already insoluble complex resulted in further inhibition of the activity. Fig. 2 shows that the extent of inhibition, as expressed by the reciprocal of the velocity, varies logarithmically with the amount of antibody protein deposited on the antigen (enzyme). Thus the inhibition of activity must be related at least in part, to the alteration or distortion of the enzyme molecule following its combination with the antibody. Teleologically this appears as a seemingly purposeful attempt by the defense mechanism to neutralize the activity of a toxin by massive assault with numerous antibody molecules when one well placed molecule is not sufficient to cover the active surface of the toxin as is the case with the antibody to the alpha toxin of *Clostridium welchii*[30].

Early in this work we observed that when the enzymic activity of alcohol dehydrogenase appeared to be maximally inhibited by the addition of a considerable excess of antibody from a given antiserum, further inhibition could nevertheless be obtained by addition of a second antiserum obtained from a more highly immunized animal, *i.e.*, a rabbit that had received a greater number of inoculations and had shown a higher antibody content in its serum. This suggested that the antibody in the second antiserum must differ significantly from that in the first since the activity of the enzyme could not be inhibited maximally by the first antiserum despite the presence of excess antibody. It appears therefore, that while the first antiserum could deposit a certain type of antibody on the enzyme to form the insoluble complex, the second could deposit a different type of antibody, not present in the first, the latter being formed in response to more intensive and more prolonged immunization of the animal. By contrast, when the order was reversed enzyme rendered maximally inhibited by the additions of excess amounts of the more potent antiserum, no further inhibition could be observed by subsequent treatment with the first. This set of observations led to the present concept of antibody-antigen interaction.

This concept requires that the following conditions be met.

1. *The antibody-antigen complex should be antigenic.* This was shown to be the case in three rabbits, each receiving two 10 mg injections of a complex obtained from a rabbit immunized by five weekly injections of the enzyme. The resulting antiserum did not inhibit the activity of the enzyme during the first 2–3 minutes of incubation but it showed immediate inhibition of the enzymic activity of the complex. Furthermore, turbidity appeared only after a lag period of about 2 minutes when the enzyme was used, whereas an immediate increase in turbidity occurred with the complex (Fig. 3). Protein measurements showed that 2–3 mg of anti-

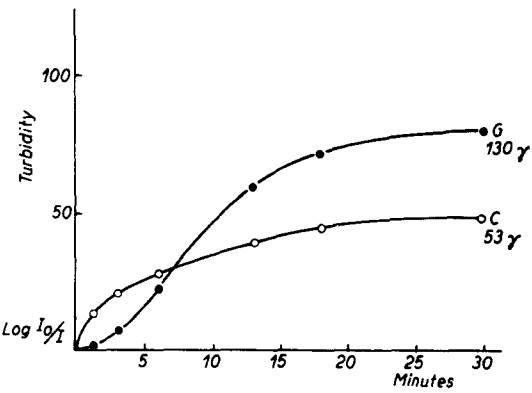

Fig. 3. Reaction of antiserum to antibody-antigen complex, with antigen (curve G) and complex (curve C). 0.7 ml of normal and immune serum were pipetted into the control and reaction cells respectively. Appropriate dilutions of the enzyme (antigen) were made in 0.1 % gelatin immediately before use. Saline-washed complex freshly prepared was suspended in the appropriate volume of saline. 0.2 ml of either enzyme or complex were added to both cells at time zero and turbidity measurements recorded at 340 mμ, pH 8.2 in the Beckman spectrophotometer. The complex was prepared from pooled sera of rabbits immunized with five injections (see text).

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body were precipitated per mg of complex in 2 hours at 20°C. In the light of the experiments cited below, it appears that the delayed reaction with the unmodified antigen (enzyme) may be due partly to cross reaction with the antibody to the complex and partly to a reaction with specific antibodies to the antigen (enzyme) formed in response to a small amount of enzyme that dissociated from the complex within the animal. Many attempts to detect any measurable dissociation *in vitro* were negative.

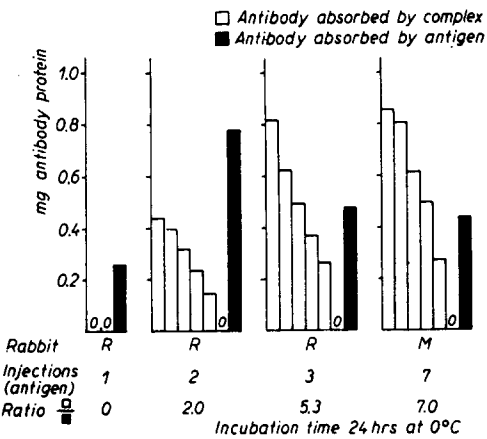

Fig. 4. Selective absorption of antibodies from various antisera by antibody-antigen complex □ and by antigen ■. Antisera obtained from rabbit R one week after first, second and third injections of antigen (enzyme) and from another rabbit M after the seventh injection are shown. Complex was prepared from pooled sera of two rabbits after the fifth and seventh injections. 1 mg of the complex was used for each successive absorption and 0.12 mg of enzyme representing antigen excess was used in the final step. Incubation at pH 8.2, 24 hours at 0° C for maximum precipitation. Control normal serum was run simultaneously (see text).

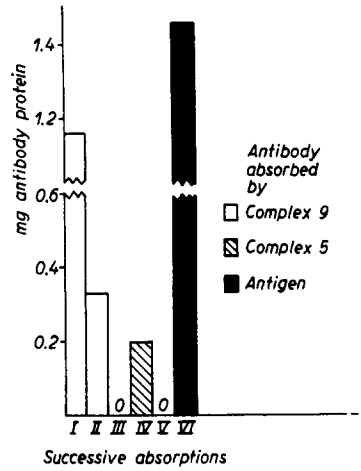

Fig. 5. Showing that an antiserum completely absorbed with one antibody-antigen complex may nevertheless contain antibody for a second antibody-antigen complex, and that the supernatant fluid then contains antibodies reactive only with the simple antigen (enzyme). Antiserum was absorbed by three successive additions of 1.0 mg each of complex 9 and two additions of 0.6 mg each of complex 5. The complexes were prepared from sera of rabbits immunized with 9 and 5 injections respectively. Incubation time, two hours at 30° C followed by one hour at 0° C, pH 8.25. Antigen was added in excess. The amount of antigen (enzyme) precipitated when antigen was finally added was calculated from the specific activity of the enzyme added minus that remaining in the supernatant fluid.

2. If an antibody to a particular complex is formed it should be possible to absorb it specifically by using the appropriate complex. Fig. 4 illustrates an experiment in which protein measurements were made on the washed precipitates. It shows that it is possible to absorb successively and exhaustively the antibody to a chosen complex and leave behind antibodies that react with the original antigen. The reaction was allowed to reach equilibrium at 0°C for 24 hours. Other experiments in which equilibrium was established at 30°C for 2 hours gave comparable results. With some antisera exhaustive absorptions were made by one complex followed by another. Fig. 5 shows that it is possible to absorb selectively with two complexes leaving sufficient antibody behind to react with the enzyme antigen. Similarly, after immunization with egg albumin (Fig. 6) it was possible to absorb some antibodies exhaustively with a complex prepared after six injections of antigen, and also leave behind anti-

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bodies that react with the antigen. However, nearly all of these latter antibodies could also be absorbed by an earlier complex prepared from an antiserum obtained after the third inoculation. It appears then that antibodies to early complexes can cross-react with the antigen. This was not the case with antisera obtained following immunization with yeast alcohol dehydrogenase, presumably because the latter was a much more antigenic substance. Measurements of antibody combination with complex or antigen were also possible in relative quantitative terms through the use of turbidity measurements. Fig. 7 shows that, under these conditions, it was possible to add successive amounts of the complex until no further increase in turbidity was observed. At this point complete absorption of the specific antibody occurred. When finally the enzyme was added it reacted with its own antibodies to form early complexes which in turn absorbed their respective specific antibodies.

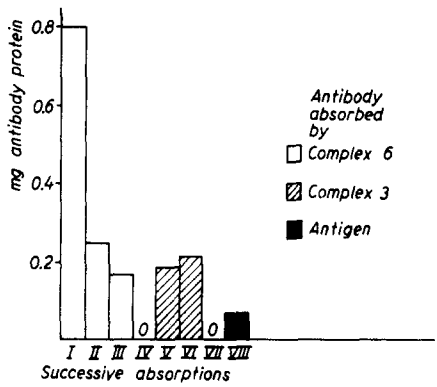

Fig. 6. Absorption of antibodies by antibody-egg albumin complex. Absorption studies were made on 1 ml of antiserum obtained from a rabbit injected with six weekly injections of egg albumin. Complex 3 and 6 were prepared from other rabbit sera obtained after 3 and 6 injections respectively. 1.2 mg of complex 6 and 0.7 mg of complex 3 were used for each successive absorption. Incubation time was 2 hours at 30° C followed by 1 hour at 0° C. Other details as in text.

We have been unable to show interaction between enzyme and complex when both were added to normal non-immune sera, either by turbidity measurements or by protein analysis of washed precipitates. Increase in turbidity upon adding the enzyme therefore, can only be due to combination with the antibody. Furthermore, identical additions were made to control samples containing normal serum. Any change therefore, that might affect the state of the complex arising from any factor in serum other than antibody was automatically eliminated. This includes any possible parcticipation of complement.

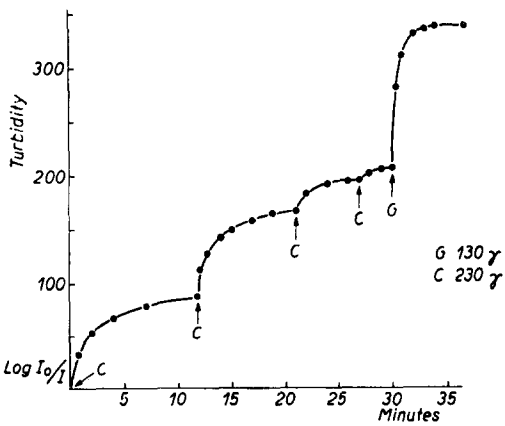

Fig. 7. Reaction of antiserum to antigen with complex (*C*) and antigen (*G*). The antiserum was obtained from a rabbit immunized with 5 injections of alcohol dehydrogenase. The complex was prepared from serum of another rabbit which also received 5 injections. Complex (*C*) 0.23 mg and antigen (*G*) 0.13 mg representing excess were added at the arrows. Details as in Fig. 3.

3. The amount of antibody to a given complex should increase with the length of exposure to the antigen and consequently to the number of injections of the antigen. Fig. 4 represents three such experiments and shows that after one injection no antibody was precipitated on a particular complex. However, after the second and third injections a considerable amount of antibody was absorbed on the complex (amounting respectively, to 1.50 and 2.6 mg of protein per ml of serum) employing

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the same rabbit for the successive weekly injections of antigen. On the other hand, the remaining antibody which was absorbed on the antigen declined after the second

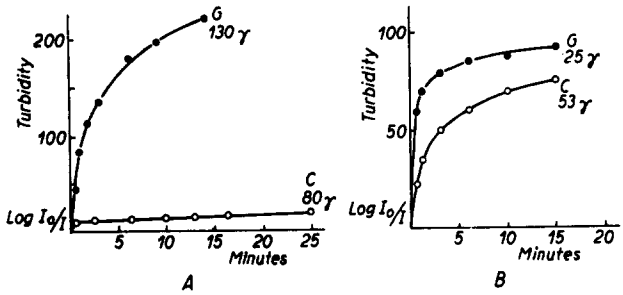

Fig. 8. Effect of the number of injections of antigen (enzyme) on the type of antibody appearing in the serum as shown by its reaction with complex (*C*) and antigen (*G*). A. antiserum obtained from a rabbit immunized with one injection; B. serum from the same rabbit after a second injection. Other conditions as in Fig. 3.

and third injection to a level of about 0.8 and 0.5 mg, respectively. Turbidity studies added further support to the above results. There was no increase in turbidity when a complex prepared from rabbits immunized with five-weekly injections was added to serum from a rabbit after one injection of the antigen, although addition of the antigen (enzyme) to the serum produced immediate increase in turbidity (Fig. 8A). However, after the same rabbit received a second injection of antigen a considerable increase in antibody to the complex resulted (Fig. 8B).

4. Since there may be a high degree of specificity of the antibodies formed to the antigen and to the complexes, the kinetics of the reaction of one type of antibody with its specific antigen should be independent of the presence of the other type of antibody. By measuring reaction rates it could be shown that after exhaustively absorbing the specific antibodies to a given complex, the rate of initial turbidity formation due to the addition of the antigen (enzyme) (Fig. 9B) was identical to that observed when the same amount of antigen was added to the same amount of unabsorbed serum under identical conditions (Fig. 9A). With some antigens the antibodies to the various complexes may show wide cross-reactions with the different complexes. In such instances, all antibodies should be fully absorbed by additions of a given complex.

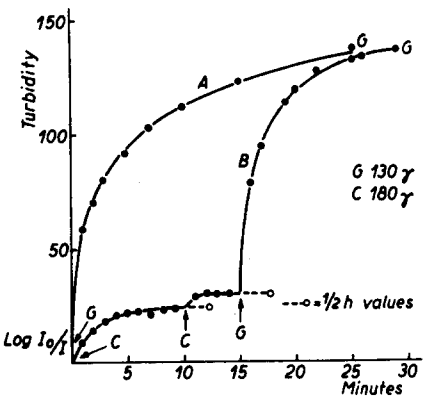

Fig. 9. Kinetics of antibody-antigen (alcohol dehydrogenase) reaction in the presence and absence of antibodies to antibody-antigen complexes. Antiserum was obtained from a rabbit immunized with two injections of alcohol dehydrogenase. Increase in turbidity was measured after adding excess enzyme at zero time (curve A). In another aliquot (curve B), complex was added two successive times followed by excess enzyme as shown by arrows. Other conditions as in Fig. 3.

5. The amount of antibody brought down per ml of a given serum with slight excess of antigen, may or may not be equal to the amount of antibody absorbed by exhaustive additions of one or more complexes, plus that

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subsequently precipitated by the antigen. The amount precipitated in excess of that brought down by the antigen alone would correspond to the amount of the so-called univalent or incomplete antibodies present. In a series of five experiments, employing successive absorptions by two different complexes followed by antigen, it was possible to show an almost exact correspondence between the sum of the antibody separately absorbed each time and the antibody precipitated when antigen was added alone to a similar aliquot of antiserum[15].

DISCUSSION

The results presented above support the view that antibodies arise in a stepwise manner in response to stepwise formation of new antibody-antigen complexes. The final product is then a composite of various single reactions each with its own characteristic dissociation constant and kinetics. Because of the inherent variability of biological responses, the response to each individual complex antigen need not be quantitatively the same in different animals or with different antigens. It is for this reason that no single mathematical expression can describe the kinetics or characteristics of this reaction, and none has been found.

A number of observations may now be easily understood on the basis of this concept. Successive immunization has been reported to show an increase in the combining ratio of antibody to antigen and a broadening of the equivalence zone[31,9,11]. These findings are quite consistent with the stepwise formation of modified complex antigens parallelling successive immunization. Furthermore, with prolonged immunization there occurs a change in antibody characteristics[9,12,32]. This is reflected by the increase of antibodies in the water-insoluble fraction of the gamma globulin and the appearance of antibodies in the a_1, a_2, and B components of the serum[9]. This fact can now be reconciled with the formation of antibodies with different physical and chemical characteristics in response to the different complex-antigens.

It has been shown[5,9,30] that so-called incomplete or univalent antibodies are present in many antibody-antigen systems. According to the concept presented above, such an antibody would have all the characteristics of an antibody to an antibody-antigen complex and is not necessarily univalent or incomplete. It should be demonstrable in many sera whenever excess antibody is formed against one or more stages of the complex. On reaction of the antibody with antigen, a series of saturated complexes would precipitate leaving the excess antibody corresponding to the particular complex in the supernatant fluid. This antibody would then precipitate in sera which produce an excess of that complex-antigen; these sera are known as precipitating sera. Furthermore, antibodies to a complex are more likely to appear in excess with repeated immunization as shown in Fig. 4, a fact that is consonant with the reported behaviour of the so-called univalent antibodies[32,33]. Finally, findings such as those obtained by CANN *et al*[34]. can readily be explained on the basis of this concept. They reported that Rh antisera fractionated by the electrophoresis convection method showed considerable loss of total precipitating antibody when each fraction was measured separately; however, upon recombination of the fractions there was complete recovery of the antibody. It is apparent that the different fractions contained limiting amounts of antibody to one or another complex-antigen. Consequently, each fraction could only form submaximal quantities of precipitate. Since the concept implies that

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the final complex obtained in an antibody-antigen reaction is the product of a series of complexes, it follows that the formation of a complex beyond a certain stage is directly dependent on the availability and the quantity of the antibody to that particular complex. Such discrepancy between fractionated and reconstituted serum is therefore predictable on this basis. We found that with antibody excess, over ten molecules of antibody (M.W. 160,000)[35] precipitated with one molecule of yeast hexokinase (M.W. 96,600)[40]. This did not represent a maximal value since the concentration of antibody was below the maximal level. Nevertheless, this amount appears to be greater than that which the antigen molecule could accommodate on its surface, even under the most favorable but unlikely assumption that the antigenic sites are properly spaced. Our concept resolves this difficulty since a second antibody (antibody II) may react with the antibody portion of a complex (antibody I).

Assuming that, under these circumstances, antibody II (which was formed against a modified site on antibody I) has some affinity for that antibody in the native state (*i.e.* as the antibody exists in antiserum and in the absence of antigen) then antibody II must exist in the serum combined in large measure with free antibody I. The result would be a structural modification of both antibodies. If the reactive site on antibody I is modified sufficiently it should then fail to react with the antigen. Antibody II would in this instance inhibit the precipitin or agglutinin reaction. This may indeed be the case with the inhibitory fractions in certain antisera identified by electrophoresis convection fractionation[36]. Addition of these fractions to other fractions containing a measured amount of antibody not only slowed down the rate of precipitate formation when antigen was added but actually resulted in diminution of the amount of the precipitate formed. It may also explain the existence of the so-called "negative" sera in cases where immunized rabbits fail to show a precipitin reaction with ordinary procedures. However, upon fractionation with convection electrophoresis, it became possible to demonstrate the formation of specific precipitates in some of the fractions[36]. These findings could be interpreted as resulting from separation of the inhibiting antibody II from its combination with antibody I. The latter then recovers its original structural configuration and can react with the antigen.

It is becoming increasingly clear that structural organization of biological systems *in vivo* may not be limited to the cell and its particulate elements such as mitochondria, chromosomes and microsomes, but actually extends to the soluble elements as well. Thus a protein *in vivo* may be different in structure from what it is as isolated, by virtue of association with other substances. Numerous instances strongly suggest this. Among these is the observation that antibody-antigen interactions in the case of certain Rh agglutinins, may fail to manifest themselves *in vitro* in the absence of a protein such as serum albumin. The albumin here may well serve to orient the reactive surface of the antibody to its antigen through protein-protein interaction. The fact that intact homologous brain tissue is not antigenic, but autolyzed homogenates of the homologous brain may be antigenic[19] may also be visualized in this light. Here the antigenic substance may have acquired an altered configuration by association with substances present in the autolyzed brain tissue.

Finally, the concept that the antibody-antigen complex becomes antigenic by virtue of the altered surface structure of its two component parts may have some clinical application. When heterologous antiserum, such as horse serum, is injected into a patient for therapeutic purposes, the antibodies generated against the horse

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serum (antigen) react with the antigen to produce the manifestations of serum sickness. The resulting tissue damage was shown to be due to antigen interaction with antibody in the tissue[37]. The complex resulting from the antibody-antigen reaction is now, according to our concept, a new antigen and after a few days should generate its own specific anticomplex antibodies. When these antibodies are released into the tissues they in turn react with the complex and would be expected to produce pathological manifestations of serum sickness. This second reaction can occur only if the complex is present when the second crop of antibodies is formed. The availability of the complex would in turn depend on the presence of the original antigen and of sufficient amount of the first crop of antibodies presumably formed against the original horse antiserum. The clinical manifestations of serum sickness have in fact been repeatedly shown to recur[26,38]. The rash may recur once or oftener. Similarly, lymph node enlargement, arthralgia, and leucocytosis may show a recurrence after abating for several days. Scarlet fever may afford a similar example. It has long been known that in this disease some signs or symptoms may recur in a cyclic manner also with an interval of several days[38,39]. In this instance the first symptoms may be due to tissue damage caused by the toxins. Subsequent manifestations may be explained through the reaction of the first crop of antibodies with the antigen followed by that between the subsequent crops of antibodies with their respective specific antibody-antigen complexes. The frequency of such recurrences would be governed by the availability of subsequent complexes as well as the magnitude of their antigenicity, both of which would be expected to diminish with time. These recurrences heretofore have been attributed to the multiple antigens present in horse sera or produced during the bacterial infection in scarlet fever.

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SUMMARY

1. By selective absorption of rabbit antisera to crystalline yeast alcohol dehydrogenase and crystalline egg albumin as antigens it is possible to show specific antibodies to antibody-antigen complexes and to the antigen.

2. Antibody-antigen complexes show an antibody response when injected into rabbits. Such antibodies react more rapidly with the complex than with the antigen.

3. Rabbits injected once with antigen show little or no antibody to a chosen antibody-antigen complex. Rabbits injected two or more times show progressively more antibody to that complex.

4. On the basis of these findings a new theory of antibody-antigen reaction is proposed. Its possible role in the understanding of some aspects of immunochemistry is discussed.

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REFERENCES

1 H. Wu, L. H. Cheng and C. P. Li, *Proc. Soc. Exptl. Biol. Med.*, 25 (1927) 853.
2 H. Wu, P. P. T. Sah and C. P. Li, *ibid.*, 26 (1928) 737.
3 M. Heidelberger and F. E. Kendall, *J. Exptl. Med.*, 50 (1929) 809.
4 S. Arrhenius, *Quantitative Laws in Biological Chemistry*, London, 1915.
5 M. Heidelberger and F. E. Kendall, *J. Exptl. Med.*, 61 (1935) 559, 563.
6 A. D. Hershey, *J. Immunol.*, 45 (1942) 39.
7 L. Pauling, D. Pressman and C. Ikeda, *J. Am. Chem. Soc.*, 64 (1942) 3010.
8 M. Heidelberger, *Bacteriol. Revs.*, 3 (1939) 49.
9 Topley and Wilson, *Principles of Bacteriology and Immunity*, Vol. I, Edward Arnold and Co., London, 1948, 3rd. ed.
10 W. C. Boyd, *Fundamentals of Immunology*, Interscience Publishers, New York, 1947.
11 E. A. Kabat and M. M. Mayer, *Experimental Immunochemistry*, Charles C. Thomas, Springfield, Ill., 1948.
12 E. Racker, *J. Biol. Chem.*, 184 (1950) 313.
13 V. A. Najjar and J. Fisher, *Bull. Johns Hopkins Hosp.*, 96 (1955) 165.
14 V. A. Najjar and J. Fisher, *Proc. Soc. Clin. Invest.*, 47th annual meeting, May, (1955) p. 49.
15 V. A. Najjar and J. Fisher, *Science*, 122 (1955) 1272.
16 K. Landsteiner and S. Simms, *J. Exptl. Med.*, 38 (1923) 127.
17 E. A. Burky, *J. Allergy*, 5 (1934) 466.
18 F. F. Schwentker and F. C. Comploier, *J. Exptl. Med.*, 70 (1939) 223.
19 F. F. Schwentker and T. M. Rivers, *J. Exptl. Med.*, 60 (1934) 559.
20 W. Burrows, *Bacteriology*, W. B. Saunders and Co., Philadelphia, 15th edition, 1950, p. 280.
21 J. Furth, *J. Immunol.*, 10 (1925) 777.
22 D. H. Campbell and F. H. Johnson, *J. Am. Chem. Soc.*, 68 (1946) 725.
23 F. H. Johnson and G. G. Wright, *Proc. Natl. Acad. Sci. U.S.*, 32 (1946) 21.
24 S. B. Hooker, *J. Immunol.*, 9 (1924) 7.
25 W. H. Park, *J. Immunol.*, 9 (1924) 17.
26 W. T. Longcope, *Medicine*, 22 (1943) 251.
27 F. H. Johnson, H. Eyring and M. J. Polissar, *The Kinetic Basis of Molecular Biology*, John Wiley and Sons, New York, 1954.
28 O. H. Lowry, N. J. Rosebrough, A. Farr and R. J. Randall, *J. Biol. Chem.*, 193 (1951) 265.
29 E. A. Krebs and V. A. Najjar, *J. Exptl. Med.*, 88 (1948) 569.
30 M. G. Macfarlane and B. C. J. G. Knight, *Biochem. J.*, 35 (1941) 884.
31 M. Heidelberger, H. P. Treffers and M. Mayer, *J. Exptl. Med.*, 71 (1940) 271.
32 R. R. Race, R. Sangers and J. G. Selwyn, *Nature*, 166 (1950) 520.
33 A. S. Wiener, *Ann. Allergy*, 6 (1948) 293.
34 J. R. Cann, R. A. Brown, J. G. Kirkwood, P. Sturgeon and D. W. Clarke, *J. Immunol.*, 68 (1952) 243.
35 E. A. Kabat, *J. Exptl. Med.*, 69 (1939) 103.
36 J. R. Cann, D. H. Campbell, R. A. Brown and J. G. Kirkwood, *J. Am. Chem. Soc.*, 73 (1951) 4611.
37 C. V. Hawn and C. A. Janeway, *J. Exptl. Med.*, 85 (1947) 571.
38 C. F. Von Pirquet and B. Schick, *Die Serumkrankheit*, Franz Deuticke, Leipzig, 1905.
39 G. Fanconi, *Scharlachproblem, Abhandlungen aus der Kinderheilkunde und ihren Grenzgebieten*, Heft 13, S. Karger, Berlin, 1926.
40 M. R. McDonald in S. P. Colowick and N. O. Kaplan, *Methods in Enzymology*, Vol. I, Academic Press, Inc., New York, 1955, p. 269.

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