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NATIONAL ACADEMY OF SCIENCES CONFERENCE ON COMPLEMENT

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In the spring of 1950 the writer undertook to organize a conference, to be sponsored by the National Academy of Sciences, on some phase of immunology. As a result of discussions on suitable topics it was decided to assemble a group, with a wide diversity of interests, in order to throw light from all sides on the subject of *complement.*

The reasons for the choice of topic were as follows:

Following the introduction of rigorous, quantitative analytical micromethods for the estimation, in weight units, of antigens and antibodies the part played by these substances and their modes of interaction in immunity to infectious disease became greatly clarified.[1] Much progress has resulted from the application of similar methods to the study of complement,[2] which, next to antigens and antibodies, is the most important auxiliary substance concerned with immunity. However, the complexity of complement and its associated phenomena frequently made difficult the interpretation of analytical data.

Often the mere combination of antibodies with the infectious agent is insufficient to set in motion the biological events which terminate a disease. It is under these circumstances that complement plays its most important role, for it promotes phagocytosis of infectious agents which have combined with antibody and in many instances it mediates their dissolution, or lysis. Not only is complement of great interest in this connection, but also because of its enormous use in diagnostic tests such as the Bordet-Wassermann test for syphilis and numerous tests for viruses which are pathogenic for man and animals. The mechanism of complement uptake or "fixation" in these commonly used and highly crucial tests is still poorly understood, as is also the mechanism of the lysis (hemolysis, in this case) of the red cell-anti-red cell complex used as "indicator" for the presence of complement in the tests.

A conference on complement was accordingly held at Ram's Head Inn, Shelter Island, N. Y., from June 12 to 15, 1950. Those who participated were: A. R. Dochez, H. Eagle, F. Haurowitz, M. Heidelberger, C. W. Hiatt, J. F. Kent, M. H. Loveless, D. A. MacInnes, C. M. MacLeod, F. Maltaner, M. M. Mayer, S. Ochoa, A. G. Osler, A. M. Pappenheimer, Jr., L. Pillemer, E. Ponder, V. R. Potter, C. E. Rice, W. H. Seegers, and A. J. Weil. O. T. Avery, E. E. Ecker, J. W. Fisher and S. B. Hooker were unable to attend.

The topics of the tentative program were: (1) Chemistry of complement and its components. (2) Complement fixation and its relation to antigen-antibody reactions, including neutralization of viruses. (3) Immune hemolysis: kinetics, mechanism, relation to enzymatic processes. (4) Bacteriolysis; enhancement of phagocytosis, immobilization of spirochetes. (5) Complement and the coagulation of the blood.

Actual discussion developed somewhat as follows:

1. Definition of Complement (C').—Particularly for the benefit of those present who had not actually worked with C' an attempt was made to define C' but agreement could not be reached. This was mainly because independent, functionally different methods of measurement lead to conflicting results. For example, the diagnostically important "fixation" of C', that is, its uptake by the interaction of antigen and antibody, is measurable as a first approximation by the increase in weight of the antigen-antibody complex.[2] The values so obtained, however, do not run parallel to the decrease observed in the hemolytic power of C',[3] the biological property of C' actually measured in the diagnostic test. The importance was emphasized of studies now under way to resolve this discrepancy. It was, however, generally agreed that Muir's old definition, "a fixable constituent of fresh serum"[4] required supplementation by something like "C' is a complex of serum constituents which brings about the lysis of red cells, immobilization of spirochetes, the killing of certain Gram-negative bacteria and the opsonization of certain bacteria, all of which functions usually require the cooperation of antibodies to the cells involved." Other activities and manifestations of C' were discussed but not included in the definition because of lack of understanding of the underlying phenomena.

2. Chemistry of C'.—Pillemer gave a brief résumé. Of the four components of C' ($C'1$, $C'2$, $C'3$, $C'4$) only $C'1$ has thus far been isolated, from guinea pig C', in reasonably pure form as judged by electrophoretic and ultracentrifugal analysis.[5] This work has had far-reaching consequences and the urgency of the isolation of the other components in a state of purity was discussed by the group. Heidelberger told of the separation of $C'3$ as a single activity from pig C', which is usually characterized by a titer of $C'3$ tenfold that of $C'3$ in guinea pig C'.

3. Measurement of C' *by Increase in Weight of Specific Precipitates.*—The technique and limitations of the method were outlined by Heidelberger. Guinea pig, human, bovine and pig complements all give values of C' nitrogen between 0.04 and 0.06 mg. per ml. when volumes of C' used are extrapolated to 0. No further light was thrown on the "solubility effect" causing lower values per ml. with larger volumes of C'. It was generally agreed that most of the weight added by C' is derived from $C'1$.

4. Complement Fixation as Studied by Measurements of Hemolysis.—Recent quantitative studies were described by Osler. While these lead to

curves of C' uptake greatly resembling those of specific precipitation at far higher concentrations of antigen and antibody, the maximum C' uptake indicated by the residual hemolysis may be much greater than can be accounted for on a weight basis if an attempt is made to correlate C' weights and hemolytic titers. Heidelberger presented evidence indicating that because of preferential fixation of $C'1$ and $C'4$ from guinea pig C' measurements made before and after fixation might not be comparable owing to a shift in the limiting components, i.e., those present in lowest titer. These are always $C'2$ and $C'3$ in whole guinea pig C'. It was decided that further work was necessary for general acceptance of this view and that truly quantitative methods for the measurement of component activities were needed since the qualitative methods now available involve large uncertainties. Moreover, fixation of C', as pointed out by Mayer, might be accompanied by an inactivation of C' as well as a loading of C' molecules onto antigen-antibody aggregates.

5. *Mechanism of Hemolysis by Antibody and C'.*—Mayer reviewed some of the older work which suggests that the hemolytic process may be enzymic. Two sets of observations stand out: Mg^{++} is an essential ion[6] and as few as 50 molecules of antibody and 20,000 molecules of C' (a highly uncertain value) are required for lysis of one cell, contrasted with about 10^9 molecules of a surface-active agent, such as saponin. He then described recent kinetic studies[7] which suggest that hemolytic antibody is the enzyme and that C' is an accessory factor used up in the process, perhaps as an energy donor. The experiments have indicated that the antibody-red cell union is reversible, making possible transfer of antibody from cell to cell. This finding is compatible with the view that the antibody is the enzyme, but is not compelling since some simple lysins can undergo transfer as pointed out by Ponder. Mayer has found that with complement in excess, and with antibody the limiting factor, the velocity of lysis is inversely proportional to the total cell concentration (lysed and unlysed). On the other hand, in a system with limited complement and excess antibody, the velocity of lysis is independent of the cell concentration. It is assumed that the lysis of a red cell results from damage to its surface structure and that a cell lyses when damage becomes critical. Therefore, the maximal rate of lysis should be directly proportional to the rate of production of damage (or strain) at the surface of the cell. (This is an approximation; strict analysis involves probability theory). From the rate of damage production in the whole system $\left(\frac{dS}{dt}\right)$ one obtains the rate of damage production per cell by dividing by the total number of cells; the maximal rate of lysis would then be: $-\frac{dE}{dt} = K\frac{(dS)}{(dt)} \cdot \frac{1}{E_0} \cdot E$, where E_0 = total cells and E = unlysed cells. Accordingly, if antibody is considered to be the en-

zyme, and conditions are such that antibody is limited (i.e., substrate is in excess), the enzyme is working at top speed; therefore, variations of substrate concentration (i.e., cells) do not affect the rate at which damage is produced, but do affect the maximal rate of lysis in inverse proportion. On the other hand, when antibody as the enzyme is in excess, the rate of damage production will be proportional to E_0, and therefore the maximal rate of lysis will be independent of the cell concentration. Since these kinetic deductions are verified by experimental results obtained with hemolytic antisera exhibiting relatively rapid transfer of antibody from cell to cell, they constitute an argument that the antibody acts like an enzyme, and complement is a substance or factor which is used up.

After returning to the University of Wisconsin, Potter discussed the kinetics of hemolysis with Alberty, who had worked out a mathematical treatment of a somewhat analogous but simpler situation involving the reactivity of ϵ-amino groups on proteins. The discussion resulted in the formulation of a theory of immune hemolysis by Alberty and Baldwin. This will probably be published after the conclusion of correspondence with Mayer.

On the last day of the conference Ponder agreed, with the help of R. T. Cox, to attempt the preparation of a clear and comprehensive statement about the various schemes which have been proposed for the kinetics of hemolysis. There has also been considerable correspondence between Ponder and Alberty, as Ponder conceives of Alberty's theory as a special case of the more general theory which he and Cox expect soon to publish.

In connection with the enzyme function Pillemer pointed out that earlier studies with Ecker indicated that $C'3$ is not used up in hemolysis[8] and that he believes for this reason that $C'3$ is the enzymic constituent of this system. Haurowitz considered it possible that an antibody-complement complex might have enzymic functions.

6. *Relation of C' to Phenomena of Blood Coagulation.*—Seegers reviewed the present status of the mechanism of blood coagulation and expressed the belief, to which Pillemer agreed, that prothrombin is not identical with $C'1$. However, accelerator globulin and C' components may be related and this deserves further exploration.

Maltaner reported on the effects of cephalin on blood coagulation and C' activity,[9] but without any clear evidence that these processes are directly related.

General discussion followed of the role of Ca^{++} and Mg^{++} in blood coagulation and C' activity. The two systems differ in that Ca^{++} appears to be the crucial constituent of the blood clotting system, whereas Mg^{++} is the more important ion for the lytic activity of C'.

7. *Conglutination.*—Rice reported some of her recent work. The conglutination reaction is often useful in diagnostic tests involving bird and animal

sera. It involves the agglutination of sheep erythrocytes by heated bovine serum and fresh horse serum and can be used as an indicator system in the complement fixation test since it shows analogies to hemolysis. The heated bovine serum contributes a substance called conglutinin while the fresh horse serum is thought to contribute C' or some of its components. If a specific antigen-antibody reaction occurs it fixes the horse C' and thus prevents conglutination. Several members of the group challenged the view that the action of the fresh horse serum is due to complement, so that further study of this complex subject was indicated.

The conference adjourned with all members much stimulated by the contacts made and by the uninhibited discussions. These reflected approaches to the subject from many entirely different points of view and usually centered less about what is known of C' than about the defects and inconsistencies in the information presently available. Ways and means were discussed in detail for obtaining some of the most urgently needed data. As Haurowitz remarked, all of the participants will in future consider C' from more critical and more objective viewpoints.

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[2] Heidelberger, M., *J. Exp. Med.*, **73,** 681 (1941); Heidelberger, M., and Mayer, M. M., *Ibid.*, **75,** 285 (1942); Bier, O., *J. Immunol.*, **51,** 147 (1945).

[3] Osler, A. G., Mayer, M. M., and Heidelberger, M., *J. Immunol.*, **60,** 205 (1948); Heidelberger, M., and Mayer, M. M., *Advances in Enzymology*, **8,** 71 (1948).

[4] Muir, R., *Studies on Immunity*, Oxford Univ. Press, London, 1909.

[5] Pillemer, L., Ecker, E. E., Oncley, J. L., and Cohn, E. J., *J. Exp. Med.*, **74,** 297 (1941).

[6] Mayer, M. M., Osler, A. G., Bier, O., and Heidelberger, M., *Ibid.*, **84,** 535 (1946).

[7] Mayer, M. M., Croft, C. C., and Gray, M. M., *Ibid.*, **88,** 427 (1948).

[8] Pillemer, L., Seifter, S., and Ecker, E. E., *Ibid.*, **74,** 421 (1942).

[9] Wadsworth, A. B., Maltaner, F., and Maltaner, E., *J. Immunol.*, **30,** 417 (1936).