Meetings

Complement and Hemolysis

A two-day workshop on complement (*1*) was held at the National Institutes of Health, Bethesda, Maryland, on 28 February and 1 March 1963, to discuss progress made since the 1956 conference at Walter Reed Army Medical Center. Many of the advances have come about by using cellular intermediate products rather than the reagents R_1, R_2, R_3, and R_4, by using partially purified C′ components, and by using assay methods in which immune hemolysis is assumed to be a one-hit process.

It was agreed to reserve the standard symbols, such as C′1, C′2, C′3, and C′4 to designate the complement components as they exist in serum. Components which undergo a change from a nonhemolytic to a hemolytically active form ("activation") are referred to by adding the letter "a" to the symbol, for example, C′1 → C′1a. Letters such as "r," "s," and "t" should be assigned to new C′ components, the position of which is uncertain in the action sequence.

The first component of C′ was discussed by I. Lepow (Western Reserve University). Fractions of human euglobulin obtained by chromatography on diethylaminoethyl-cellulose (DEAE-) in the presence of trisodium-ethylenediaminetetraacetate (EDTA) were designated as C′1q, C′1r, and C′1s in the order of elution. All three fractions are necessary for the conversion of EA to EAC′1 or EAC′4 to EAC′1,4. The factor C′1q is thought to be identical with the 11*S* component previously described by Müller-Eberhard and Kunkel, and by Taranta, Weiss, and Franklin; C′1r is a hitherto undescribed component; and C′1s is believed to be C′1 proesterase. All three are required for activation of C′1 proesterase to C′1 esterase. Evidence was also presented that C′1 exists as a macromolecular complex which can be resolved into three factors by chromatography in the presence of EDTA. Once separated, these components must react first in the fluid phase to produce a molecule capable of converting EA to EAC′1.

T. Borsos (NIH) presented data on the reaction between EAC′4 and C′1 when guinea-pig reagents are used. The interaction of C′1 from untreated whole serum or partially purified C′1a and SAC′4 is rapid and almost temperature independent. The reaction, however, is complicated because C′1a transfers from site to site and from cell to cell, thus making it impossible to test whether, in addition to transfer or cycling in the presence of C′2, C′1 is converted to C′1a. Transfer was virtually eliminated by reducing the ionic strength of the diluent (see also, Rapp). It was concluded that SAC′1,4 is activated to SAC′1a,4 in the absence of serum factors at a rate which increases with temperature and that SAC′1a,4 is the intermediate product which reacts with C′2. Activation of C′1 to C′1a has been demonstrated for the first time in the hemolytic system.

Methods for separating guinea pig C′1 and C′4 were discussed by Oikawa (K. Oikawa and R. Nelson, Howard Hughes Medical Institute). These two components were eluted from a DEAE-cellulose column to which was applied guinea pig serum treated at 37°C with zymosan and at 0°C with small amounts of cellulose. Zymosan treatment depletes C′3c, which is eluted near C′1, and also C′3b, which is eluted near C′4. Absorption with cellulose removes what is presumed to be natural antibody reactive with cellulose which may bind C′1 on the column.

L. Hoffmann (Johns Hopkins University) discussed methods for measuring guinea pig C′4. Exposure of EAC′1a,4 with few SAC′4 to high concentrations of C′2 results in a steady state with respect to the number of SAC′1a,4,2a plus SAC′4,2a; this number should equal the number of C′4 molecules. Under these conditions the reaction rate between SAC′1a and C′4 yielding SAC′1a,4 depends on the concentrations of both SAC′1a and C′4 and the end point is proportional to the concentration of C′4. However, the number of SAC′1a,4,2a increases with increasing concentration of C′1a because C′1a dissociates. The discovery of Borsos and Rapp that dissociability of C′1a is reduced at low ionic strength provides the basis for a suitable C′4 assay.

H. Müller-Eberhard (Rockefeller Institute) reported that the purification of C′4 activity of human serum resulted in the isolation of a highly homogeneous β1E-globulin. Both this globulin and C′4 activity were in the same chromatographic, electrophoretic, and ultracentrifugal fractions. Uptake of β1E occurred with EAC′1 but not with EA and EAC′1 treated with diisopropyl fluorophosphate, suggesting that β1E represents human C′4. About 300 molecules of β1E per cell were needed to produce significantly reactive EAC′1a,4 from EAC′1a. Since not all of these molecules were bound and since of those bound not all were attached to SAC′1, the number of specifically bound molecules is considerably smaller than 300 per cell.

M. Mayer (Johns Hopkins University) reported that the reaction between SAC′1a,4 and guinea pig C′2 proceeds in two steps. The reactants form a dissociable complex, SAC′1a,4,2; this decomposes to SAC′1a,4,2a, which reacts with the C′3 factors, and to C′2i, a hemolytically inactive fragment detectable with antibody to C′2. The rate of the second step is highly temperature dependent. It is likely that C′1a is an enzyme which cleaves C′2 and transfers the C′2a group to a receptor on C′4. The destruction of C′2 by free C′1a in the fluid phase also yields C′2i and may be a hydrolysis competing with the C′2a transfer mediated by cell-bound C′1a. At low ionic strength (0.06; Rapp and Borsos) almost all C′1a is cell-bound and destruction of C′2 in the fluid phase is negligible; nonetheless, production of C′2i accompanies formation of SAC′1a,4,2a at low ionic strength. This proves that C′2i is formed at the cell surface. By analogy, Mayer also speculated that the well-known destruction of C′4 by free C′1a in the fluid phase might be a hydrolysis and that C′1a might mediate the union of C′4 to a receptor at the cell surface, and then the union of C′2a with C′4.

R. Stroud (Johns Hopkins University) and K. Austen (Massachusetts General Hospital) presented kinetic studies on the relation between SAC′1a,-

4,2a formation by enzymatic action of cell-bound guinea pig C′1a and the esterolytic action attributed to C′1a. Diisopropyl fluorophosphate blocks formation of SAC′1a,4,2a and C′2i. There is a linear relation between C′1a concentration and the initial velocity of SAC′1a,4,2a formation. Furthermore, there is a linear relation between the reciprocal of the initial velocity of formation of SAC′1a,4,2a and the reciprocal of the concentration of C′2. This is consistent with the known formation of the reversible intermediate SAC′1a,4,2, which is in equilibrium with reactants SAC′1a,4 and C′2. Evidence was presented that *p*-toluenesulfonyl arginine methyl ester is a competitive inhibitor of the formation of SAC′1a,4,2a.

Four guinea pig serum factors are required for the conversion of EAC′1, 4,2 to E*. These factors have been designated as C′3a, C′3b, C′3c, and C′3d by R. Nelson (H. Hughes Medical Institute). Each factor was obtained in functionally pure form by chromatography. Elution of 90 percent of each reactivity occurred at the following NaCl concentrations: C′3a, 0.056 to 0.065; C′3c, 0.076 to 0.080; C′3d, 0.080 to 0.10; C′3b, 0.12 to 0.21 (Nelson, Oikawa, Rommel). The factor C′3c reacts with EAC′1,4,2 resulting in an intermediate product which loses its reactivity with C′3b at a rate dependent on temperature. The resulting product is EAC′1,4,3c most of which can be restored to EAC′1,4,2,3c by purified C′2. The intermediate product EAC′1,4,2,-3c,3b is stable (K. Inoue, H. Hughes Medical Institute) and reacts with C′3a to form EAC′1,4,2,3c,3b,3a. This relatively stable intermediate product reacts with C′3d to yield E*. Both C′3a and C′3d appear to have enzymatic properties. These components are neither depleted during the hemolytic reaction nor during absorption of whole serum with immune precipitates (F. Rommel, H. Hughes Medical Institute).

W. Linscott (Scripps Clinic) reported that guinea pig C′3c is relatively heat stable and C′3a is heat labile. Both C′3c and C′3b are destroyed at low *p*H. C′3c is more sensitive to destruction by hydrazine than C′3b or C′3a and hydrazine has no effect on C′3d. At 0°C, C′3c reacts readily with EAC′1,4,2, and C′3b has virtually no reactivity with EAC′1,4,2,3c, and the interaction of EAC′1,4,2,3c,3b and C′3a is rapid.

In a comparison between human C′3 and guinea pig serum C′3, it was found that highly purified β1C globulin of Müller-Eberhard contains two C′3 factors analogous to guinea pig C′3c and C′3b (Linscott and Nishioka). In the discussion, Müller-Eberhard pointed out that β1C, purified by a modification of his original method, combines with, but does not prevent the loss of reactivity of EAC′1,4,2. With specific antibody to β1C it was found that EAC′1,4,2, which has reacted with β1C and has then lost reactivity, still contains β1C. Conversion of EAC′1,4,2 to a stable intermediate requires β1C and β1F which have physico-chemical properties similar to β1C except for a slightly different electrophoretic mobility.

By the use of selective destroyers as heat, hydrazine, and cobra venom, H. Wellensiek (Johannes Gutenberg University) demonstrated at least three factors necessary for guinea pig C′3 action. In addition, these factors were distinguishable by electrophoresis and they were separated by chromatography and precipitation methods. It was suggested that two of these factors are identical with Nelson's factors C′3c and C′3b.

K. Rother (Medizinische Polyklinik, Freiburg) has been breeding a strain of C′-deficient rabbits, many of which die during the first two weeks after birth. Sera of surviving animals do not kill *Salmonella typhosa*. Negative Arthus tests in C′-deficient rabbits can be made positive by injecting heated fractions of normal rabbit serum or pig C′ together with the test reagents.

S. Seifter (Albert Einstein Medical School) reported that, under conditions favoring nucleophilic attack of an ester, guinea pig C′4 is destroyed by hydroxylamine. Model esters were treated similarly and examined by the Lippman-Tuttle method for hydroxamate and by thin-layer chromatography. Acetates of the mono- or disaccharides as well as lecithin were readily stripped of acyl groups by hydroxylamine, which suggests that part of the C′4 molecule is an ester.

The effects of salt concentration, cell number, and hemolysin concentration on guinea pig C′ activity were discussed by H. Rapp (NIH). For studies on the effect of salt concentration, isotonic conditions were maintained by the addition of sucrose or mannitol. The total C′ activity was greatest when the ionic strength was lowered from 0.150 to about 0.055. This increase is partly due to firmer binding of C′1 to SA. Becker's observation that C′3 activity increases with decreasing salt concentration was confirmed. Reduction in erythrocyte concentration also increases C′ activity; the amount of hemolytic antibody needed to maintain the cells at optimum reactivity with C′, however, varies inversely with the red cell concentration. At relatively high concentrations of cells, optimum C′ reactivity can be achieved with less antibody than that required for complete saturation of receptor sites on the cell surface. With decreasing cell concentrations, a greater proportion of the receptor sites must be in combination with hemolytic antibody to achieve optimum C′ activity.

K. Amiraian (New York State Department of Health) found that carboxypeptidase A was a more effective enhancer of guinea pig C′ at conditions other than optimum for the hemolytic reaction. The role of the enzyme in obviating the effects of an inhibitory step before the formation of EAC′1,4,2 was discussed. Borsos commented that when EAC′1a,4 is treated with carboxypeptidase A, the decay rate of EAC′1a,4,2a, prepared from these cells with purified C′2, is reduced by 35 percent. There is no change in the time of maximum formation of SAC′1a,4,2a.

The need for improved and universally acceptable methods for C′ measurement was illustrated by attempts to find meaning in variations in C′ component titers of pathological sera from several species (E. Fischel, Bronx Hospital). Increase in total serum C′ activity was observed after injecting egg albumin into guinea pigs, after withdrawal of relatively large amounts of blood, and during experimental and clinical inflammation.

D. Ingram (Ontario Veterinary College) reported that in the light of Lachman's findings, he now believes that immunoconglutinin is anti-C′3 and not anti-C′4 as was thought earlier.

A committee was chosen to correlate the results obtained in different laboratories and to decide on mutually acceptable number symbols for the various C′3 factors. The symbols C′a and C′b were assigned by Rapp (1958) to two factors (obtained by DEAE-cellulose chromatography) required for the conversion of EAC′1,4,2 to E*. Subsequently it was found that C′a contains two factors. The symbol C′a was retained for the factor which was eluted at the lowest ionic strength and C′c was assigned to the factor which was eluted between C′a and C′b. It is not certain whether these three factors correspond

to C'3a, C'3c, and C'3b described by Nelson *et al.* An additional factor, C'3d, has also been described. This terminology, therefore, is complicated because it reflects both the behavior of these factors on DEAE-cellulose chromatography and the order in which they were discovered. Thus, the symbols bear no relationship to the sequence of action of the various factors. The committee was charged with the task of resolving these nomenclature problems on the basis of experimental evidence. It was agreed that the present symbols should be replaced by C'3, C'5, C'6, and C'7 when the members of the committee reach agreement concerning the identity and sequence of action of the several C'3 factors now recognized.

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Note

1. Complement (C') is a group of naturally occurring macromolecular serum factors which interact with antigen-antibody complexes. If the antigen (S) on the surface of an erythrocyte (E) is in combination with specific antibody (A), the complex (SA), or in cellular terms, EA, can interact with C' and as a result the cell is destroyed. The C' components act in the sequence C'1, C'4, C'2, C'3. The third component (C'3) is not a single factor. EAC'1 is a complex produced by interacting EA and C'1, similarly complexes reacting further in the sequence are designated EAC'1,4, EAC'1,4,2, and so forth. A cell which has reacted with A and all the components of C' is designated E*. Cells in the state E* release their hemoglobin at a rate dependent on temperature. In molecular terms, the symbol E is replaced by the symbol S. R_1, R_2, R_3, and R_4 refer to sera so treated that C'1, C'2, C'3, and C'4, respectively, are lacking. Terms beginning with the symbol β refer to components identified by immunoelectrophoretic analysis. See also E. A. Kabat and M. M. Mayer, *Experimental Immunochemistry* (Thomas, Springfield, Ill., ed. 2, 1961).

Thirst: Regulation of Body Water

Scientists representing many disciplines focused their attention on a common problem, water intake in the regulation of body fluids, at a conference held at Florida State University, Tallahassee, 1–3 May.

In the opening address E. R. Adolph (Rochester) discussed terminology, quantitative relations between water deficit and drinking, the history of these concepts, and the evolution of modern experimental methods. As there is no "unique stimulus" for drinking, Adolph emphasized the need to specify and determine the relationships between the multiple factors related to drinking and the regulation of volume and dilution in the animal body. The emphasis on the remarkable differences in water intake between species and among individuals was amplified by Hudson's (Rice) comprehensive treatment of water regulation in desert mammals and Cade's (Syracuse) analysis of water and salt balance in granivorous birds. The multiple-factor concept of the determination of water intake was confirmed by much of the evidence presented.

Minimal water requirements under conditions of heat and work were discussed by Henschel (U.S. Public Health Service, Cincinnati, Ohio). Although difficult to determine because of differences in individuals, conditions of work, and environment, considerable data are available and specific recommendations can be made for a variety of situations. Several physical and chemical methods have been developed to provide an adequate supply of water under unusual environmental conditions, such as shipwreck at sea or confinement for prolonged periods in an enclosed vehicle (Sendroy, U.S.N. Medical Research Institute, Bethesda). Interactions of water, food, and temperature regulation in the monkey during short-term heat and cold stress were described by Hamilton (Veterans Administration Hospital, Coatesville, Pennsylvania).

Holmes (Colorado) demonstrated that thirst is still a serious consideration in clinical medicine where the oral factors are important determinants of fluid intake. Interactions among osmotic pressure, salivary flow, vasopressin, plasma volume, and water intake were emphasized by Towbin (VA Hospital, Little Rock). He also speculated on the role of specific "taste" afferents in the determination of fluid intake. Towbin's discussion of the role of gastrointestinal factors in the absorption of water and satiety was followed by Jacobs (Illinois, Urbana) who reported on the experimental separation of oral and gastric factors in water-food ingestion in the rat. An interesting analysis of psychogenic polydipsia emphasized that without proper tests this condition can be confused with diabetes insipidus (Falk, Michigan). Effects of propylthiouracil, thiouracil, and methimazole