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Chemical Analysis of the C'1 Fraction of Human Complement.*

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Although the chemical and biological functions of the 4 components of human complement have been studied in considerable detail,[1-6] little is known of the composition and structure of the individual components. The preparation of an electrophoretically homogeneous midpiece (C'1) fraction has been reported.[3] In order to characterize the constitution of this fraction of human complement, a supply of electrophoretically homogeneous C'1 was prepared and subjected to an elementary as well as an amino acid analysis.

Preparation. C'1 was separated from human serum by the method described in a previous publication of this laboratory.[3] It was obtained as a β-globulin comprising 0.6% of the total serum protein. An ultracentrifuge study of this preparation disclosed the presence of a major component comprising 70% of the total fraction. Sedimentation constant of this component was 6.9 Svedberg units referred to water at 20°C. The remaining 30% of the fraction sedimented more rapidly.

On a dry basis the preparation contained 13.9% nitrogen and after repeated ether extraction contained 15.0% nitrogen, demonstrating that approximately 10% of the

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1 Ecker, E. E., Pillemer, L., and Seifter, S., *J. Immunol.*, 1943, **47**, 181.

2 Seifter, S., Pillemer, L., and Ecker, E. E., *J. Immunol.*, 1943, **47**, 195.

3 Pillemer, L., Seifter, S., San Clemente, C. L., and Ecker, E. E., *J. Immunol.*, 1943, **47**, 205.

4 Dozois, T. F., Seifter, S., and Ecker, E. E., *J. Immunol.*, 1943, **47**, 215.

5 Seifter, S., Dozois, T. F., and Ecker, E. E., *J. Immunol.*, 1944, **49**, 45.

6 Dozois, T. F., Seifter, S., and Ecker, E. E., *J. Immunol.*, 1944, **49**, 31.

original material was ether-soluble (lipoid). The ether-insoluble portion was subjected to analysis.

Methods of Analysis. Since analytical values are usually presented on the basis of ash-free material, the ash content of the ether-insoluble portion of C'l was determined. It was found to be 1.09% exclusive of NaCl.

Carbon, hydrogen, and total sulfur analyses were performed by the microchemical methods of Pregl, and were recalculated on an ash-free basis. For these determinations, samples were first brought to constant weight by drying *in vacuo* at 70°C over phosphorus pentoxide.

For the determination of arginine, histidine, cystine, serine, and threonine, 150 to 200 mg of the protein was hydrolyzed with 20% hydrochloric acid for 24 hours. The solution was then carefully brought to pH 5.5 with sodium carbonate and diluted to volume. After 24 hours the humin which formed was collected by centrifugation, washed twice with water and analyzed for nitrogen by the Kjeldahl micro-method. In the hydrolysate, arginine and histidine were determined colorimetrically at 490 and 515 mμ respectively, by the procedure of Macpherson.[7] Cystine was determined at 640 mμ by a similar method.[8] Serine was determined in the manner described by Nicolet and Shinn,[9] and the results were increased by 10% to allow for destruction during the acid hydrolysis.[10] The method of Shinn and Nicolet[11] was used to determine threonine. A Cenco-Sheard spectrophotolometer was used for all colorimetric determinations. All analyses were run in duplicate except for arginine.

For the analysis of tryptophane and tyrosine, 75 to 150 mg of protein were hydrolyzed with 5N sodium hydroxide for 24 hours. In the hydrolysate, tryptophane and tyrosine were determined by the Folin-Lugg method[12] at 490 and 515 mμ respectively. Tryptophane was also determined by the method of Eckert[13] at 550 mμ. Since the color in the Folin-Lugg method was observed to fade 45 seconds after the solution was mixed with the reagents, it is probable that Eckert's method is more reliable.

TABLE I.
Composition of the C'l Fraction of Human Complement (Per Cent of Ash-free Material.)

	%
Carbon	52.06
Hydrogen	7.59
Nitrogen	15.30
Humin-nitrogen	0.60
Cystine sulfur	0.64
Noncystine sulfur	0.55
Total sulfur	1.19
Arginine	4.66*
Histidine	1.87
Cystine	2.41
Methionine	2.09†
Serine	7.76
Threonine	11.18
Tyrosine	4.47
Tryptophane (Folin-Lugg)	1.08
(Eckert)	1.12
Polysaccharide	4.26

* One determination only.
† Calculated from noncystine sulfur, approximate only.

In connection with some other work, one of us (B.L.) has made a thorough investigation of the Tillmans-Alt orcinol reaction with carbohydrates. Experiments have shown that protein heated with 60% sulfuric acid gives a slight yellow color which absorbs light in the same region as does the color developed by the orcinol-carbohydrate reaction. The intensity of the color increases with the protein concentration.[14] However, if 0.2 ml of the 2% orcinol reagent is added to 60% sulfuric acid before heating with protein, the solution may be heated at 80°C for 20 minutes without producing any color which absorbs light at 520 mμ.

For the determination of polysaccharides in the C'l preparation, therefore, 0.4 ml of the ether-insoluble protein solution, 0.2 ml of orcinol reagent (2% in 25% sulfuric acid)

[7] Macpherson, H. T., *Biochem. J.*, 1942, **36**, 59.
[8] Lugg, J. W. H., *Biochem. J.*, 1932, **26**, 2160.
[9] Nicolet, B. H., and Shinn, L. A., *J. Biol. Chem.*, 1941, **139**, 687.
[10] Brand, E., Kassel, B., and Saidel, L., *J. Clin. Invest.*, 1944, **22**, 437.
[11] Shinn, L. A., and Nicolet, B. H., *J. Biol. Chem.*, 1941, **138**, 91.
[12] Lugg, J. W. H., *Biochem. J.*, 1937, **31**, 1422.
[13] Eckert, H. W., *J. Biol. Chem.*, 1943, **148**, 205.
[14] Heidelberger, M., and Kendall, F. E., *J. Immunol.*, 1936, **30**, 267.

and 6.0 ml of 60% (by volume) sulfuric acid were thoroughly mixed and heated for 20 minutes at 80°C. The intensity of the color in the cooled solution was measured at 520 mμ. For a blank sample, 0.4 ml of the same protein solution was mixed with 0.2 ml of distilled water and 6.0 ml of 60% sulfuric acid and treated as described above. The error of the method is ±2.0%. Determinations were made in duplicate.

Summary. A tabulation of the elementary, (partial) amino acid, and carbohydrate composition of the preparation of C'1 is presented in Table I. The figure for hydroxy amino acid content of the C'1 preparation (23.4%) compares favorably with the value of 21.3% obtained for a β-globulin concentrate by Brand, Kassel, and Saidel,[10] and with the value of 22.5% obtained by these authors for γ-globulin.

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Preparation and Properties of Erythrocyte-free Avian Plasmodia.*

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While studying the serological specificity of avian plasmodia using hemolyzed, parasitized erythrocytes as antigen and the rabbit for the production of antiserum we confirmed the experience of previous workers[1,2] that absorption of such antisera with uninfected erythrocytes seemed to remove all agglutinative or precipitative antibodies reactive with infective erythrocytes or extracts of them. It has been suggested that the malaria parasite may contain substances antigenically similar to normal blood cell components which would explain this phenomenon.[3-5] However, this suggestion does not explain the species and even strain specificity of malarial immunity in the vertebrate host though it may be an additional factor in the reaction. In order to account for the observed absorption of antibodies by uninfected erythrocytes Eaton and Coggeshall reasoned that the complicating factor was "that the parasites cannot be completely separated from the red cells of the monkey or other animals in whose blood they multiply."[1]

Our attempts to produce better test antigens by fragmentation (grinding or freezing and thawing) of duck red cells parasitized with *Plasmodium lophurae* and *Plasmodium cathemerium* were unsuccessful. Microscopically these preparations showed only a small proportion of objects which might be called intact free parasites whether the cells were exposed for a short or a long time to such mechanical treatment. It is possible that such ground materials would be poor test antigens since some precipitin reactions can be demonstrated only after adsorption of the antigen on collodion particles. Thus, the very integrity of the parasite may be essential to the best reactivity if only as a function of the surfaces involved. The removal of the plasma membrane of the erythrocyte in such a fashion as to free whole malaria parasites then seemed the first step in the experimental attack.

The nucleus of the avian erythrocyte served as one of the best clues to the in-

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2 Zuckerman, A., *J. Infect. Dis.*, 1945, **77**, 28.

3 Heidelberger, M., and Mayer, M. M., *Science*, 1944, **100**, 359.

4 Oliver-Gonzalez, J., and Torregrosa, M. V., *J. Infect. Dis.*, 1944, **74**, 173.

5 Butts, D. C. A., *Am. J. Trop. Med.*, 1945, **25**, 417.