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Quantitative Studies of Complement Fixation.*

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The spectrophotometric method for the precise estimation of the hemolytic activity of complement[1,2] has now been applied to a quantitative study of complement fixation by the interaction of single purified antigens and homologous and cross-reacting antibodies.

As in the classical complement fixation technic, a constant amount of complement (C′) is employed. However, sufficient C′ (usually 50 50% hemolytic units) is added to avoid complete fixation. The extent of fixation is determined by quantitative estimation of the residual hemolytic activity and subtraction of this value from the mean value of the C′ activity buffer, antigen and antibody controls. The results, expressed as the number of C′ units fixed, are more readily interpretable than the indirect expressions employed by previous investigators.[3,4] This method, moreover, permits the use of a single table of conversion factors as calculated from von Krogh's equation with a value for $1/n = 0.2$.[2]

Materials and Methods. Standardized suspensions of sheep erythrocytes are prepared as in [1]. Since Ca^{++} and Mg^{++} are essential constituents of the hemolytic system,[2] these ions are supplied in optimal concentrations by the use of veronal buffer[2] containing 0.00015 *M* $CaCl_2$ and 0.0005 *M* $MgCl_2$ as diluent for all reagents used in the test.

2.5 ml† of a dilution of immune rabbit serum (in this instance antipneumococcus Type III) are mixed in the cold in 25 x 115 mm tubes with 5.0 ml of a dilution of guinea pig C′ containing 50 50% hemolytic units; 2.5 ml of S III‡ solution are then added with thorough mixing. The tubes are incubated at 37 ± 0.1°C. After 60 minutes§ the tubes are chilled in ice-water. A measured volume of the contents of each tube is diluted with chilled isotonic buffer so that aliquot portions of this dilution will cause partial lysis in the range of 20-80% and thus furnish analyses appropriate for the estimation of the 50% hemolytic unit of the residual C′. The hemolytic reaction is conducted as in [2] except that the total volume is 7.5 ml instead of 5.0 ml.

Details of procedure and calculations of a typical experiment are illustrated in Table I.

Results. 1. With a constant quantity of S III, the units of C′ fixed increase as the amount of antibody N is increased. With 0.25 μg S III, the increase in fixation is linear in the range of 0.5 to 1.5 μg of antibody N. The linear relationship might be utilized for measurements of minute amounts of antibody.

2. No fixation of C′ occurs when the ratio of antibody N to S III approaches 2 or less, in the region of extreme antigen excess.

Data have also been obtained with constant amounts of antibody and varying quantities of S III, with varying numbers of

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1 Mayer, M. M., Eaton, B. B., and Heidelberger, M., *J. Immunol.*, 1946, **53**, 31.

2 Mayer, M. M., Osler, A. G., Bier, O. G., and Heidelberger, M., *J. Exp. Med.*, 1946, **84**, 535.

3 Wadsworth, A., Maltaner, F., and Maltaner, E., *J. Immunol.*, 1938, **35**, 217, and other papers.

4 Rice, C. E., *J. Immunol.*, 1947, **55**, 1, and earlier papers.

† All measurements are made with calibrated pipettes and all glassware is cleaned with sulfuric acid-sodium dichromate cleaning mixture.

‡ SIII = specific polysaccharide of Type III pneumococcus.

§ In subsequent studies a fixation period of 90 minutes was used.

TABLE I.
Units of Guinea Pig C′ Fixed by 0.25 μg S III and Varying Quantities of Rabbit Antipneumococcus Type III Serum C-28.

Controls		Titration residual C′: Dilution of reaction mixture	Vol. of dilution tested, ml	Veronal buffer added, ml		Hemolysis %	Factor†	Residual C′, units	C′ fixed, units	C′ fixed corr. to 50 units, units
Serum (4 μg antibody N)		1 + 9	2.0	4.5	1.0 ml hemolytic system added. Incubation 60 min.* at 37°C.	45.9	0.970	48.5		
			2.5	4.0		71.8	1.202	48.1		
S III (0.25 μg)		1 + 9	2.0	4.5		49.5	0.998	49.9		
			2.5	4.0		72.8	1.215	48.6		
Buffer		1 + 9	2.0	4.5		47.1	0.978	48.9		
			2.5	4.0		71.8	1.202	48.1		
							Mean:	48.7		
Antibody N used, μg	Fixation 60 min. at 37°C.									
0.71		1 + 9	2.0	4.5		39.5	0.915	45.8	3.5	3.6
			2.5	4.0		63.3	1.113	44.5		
0.94		1 + 9	2.5	4.0		52.4	1.02	40.8	7.8	8.0
			3.0	3.5		73.9	1.23	41.0		
1.18		1 + 9	3.0	3.5		60.7	1.090	36.3	12.8	13.1
			3.5	3.0		74.9	1.241	35.5		
1.66		1 + 9	3.5	3.0		43.5	0.950	27.1	21.9	22.5
			4.0	2.5		56.9	1.056	26.4		
2.36		1 + 4	3.0	3.5		59.5	1.078	18.0	30.7	31.5
			3.5	3.0		76.0	1.260	18.0		
3.78		1 + 4	3.5	3.0		47.5	0.982	14.0	34.7	35.6
			4.0	2.5		64.4	1.125	14.0		

* In other experiments 45 min. were shown to be adequate.
† From Reference 2, based on $1/n = 0.2$.

C′ units, and with other immune systems, and these are being assembled for publication.

Summary. A quantitative method for the determination of complement fixation is briefly described and data are given for its application to one immune system.

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Histochemical Demonstration of Liver Glycogen in Human Diabetic Acidosis by Liver Biopsy.

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In spite of the extensive experimental and clinical literature which has been devoted to diabetes mellitus, few observations have been concerned with liver glycogen in human diabetes. These were based on autopsy material or on single specimens removed at operation. Two recently developed technics make the direct study of the liver glycogen content in the human feasible under clinical conditions. The 3-inch Silverman biopsy needle[1] makes it possible to obtain enough tissue for histologic examination; and Gomori's histochemical method for the demonstration of glycogen[2] appears to permit a quite accurate estimate of the glycogen content.

Method. A core of liver tissue is secured by the Silverman needle, at varying times during the treatment of patients in diabetic acidosis. Although needle biopsy is a reasonably safe procedure, it should be performed cautiously, and only after defects of the clotting mechanism have been ruled out. Moreover, the risk may be greater in unconscious patients.

The tissue, fixed for 24 hours in absolute alcohol, is stained by Gomori's method. Blood sugar levels are determined by the method of Folin and Wu,[3] and plasma CO_2 combining powers by the manometric Van Slyke technic.[4]

Results. Liver biopsies have been performed in diabetic patients with acidosis, and the histologic results correlated with the clinical and biochemical status of the patient. An example of the data so obtained is shown in Fig. 1. This patient was a 35-year-old Negro woman, not previously known to be diabetic, who had had polyuria and polydipsia for 4 months. During the 10 hours prior to admission she had been comatose. No evidence of infection was found. Fig. 1-A shows the liver glycogen prior to treatment. At this time, the blood sugar level was 358 mg/100 cc, the CO_2 combining power 27.8 vol % (11.6 mM/L) and there was 4+ glycosuria and 2+ acetonuria. Note that although the glycogen content is greatly diminished, a small amount is still present. Many large vacuoles, indicating fatty metamorphosis, are seen in the liver cells of the midzonal portions of the lobules.

Fig. 1-B shows the appearance of the liver 7½ hours later. By this time, the patient had received 300 units of regular insulin, 4000 ml saline and 300 g of glucose by vein. Her urine had been acetone-free for 2½ hours, but still showed 4+ test for sugar. The CO_2 combining power was 43.2 vol % (19.3 mM/L). Note the striking restoration of glycogen. Fat vacuoles are still numerous. A third biopsy (Fig. 1-C) was taken after 6 days, when the diabetes had been well controlled for 3 days. Note that there

1 Silverman, I., *Am. J. Surg.*, 1938, **40**, 671.

2 Gomori, G., *Am. J. Clin. Path.* (Tech. Sect.), 1946, **10**, 177.

3 Folin, O., and Wu, H., *J. Biol. Chem.*, 1920, **41**, 367.

4 Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, **61**, 523.