preparation. 3. Both acetone soluble and acetone insoluble fractions markedly decrease the muscle tension curve in 2 to 20 minutes. When the muscle becomes inexcitable on indirect stimulation, it is also inexcitable on direct stimulation. 4. The active material is heat stable, dialysable and is antagonized by $CaCl_2$ added to the extracts. 5. Concentrations of potassium 2 to 44 times the potassium concentration of frog Ringer were found in the extracts. These concentrations of potassium are sufficient to produce the muscle inhibition observed. 6. The significance of these results relevant to the search for a blocking agent in the thymus glands of myasthenic patients is discussed.

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1. McEachern, D., *Med.*, 1943, v22, 1.
2. Schweitzer, A., *J. Exp. Med. and Surg.*, 1947, v5, 264.
3. Constant, G., Porter, E. L., Andronis, A., and Rider, J. A., *Texas Rep. Biol. and Med.*, 1949, v7, 350.
4. Constant, G. A., Porter, E. L., Seybold, H. M., and Andronis, A., *Am. J. Physiol.*, 1949, v159, 565.
5. Wilson, A., Obrist, A. R., and Wilson, H., *Lancet*, 1953, v1, 368.
6. Wilson, A., and Stoner, H. B., *Q. J. Med.*, 1944, v13, 1.
7. Castleman, B., and Norris, E. H., *Medicine*, 1944, v38, 27.
8. Sereni, E., *J. Physiol.*, 1925, v60, 1.
9. Fenn, W. O., and Cobb, D. M., *J. Gen. Physiol.*, 1934, v17, 629.
10. Gellhorn, E., *Biol. Bull.*, 1930, v59, 338.
11. Solandt, D. Y., *J. Physiol.*, 1935, v84, 3.
12. Raventos, J., *Q. J. Exp. Physiol.*, 1938, v27, 99.
13. Torda, C., and Wolff, H. G., *J. Lab. and Clin. Med.*, 1947, v32, 1374.
14. Overton, E., *Pflug. Arch.*, 1904, v105, 176.

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Serum Complement in Hepatobiliary Diseases.* (22111)

EMANUEL E. MANDEL AND KURT LANGE.

Chicago Medical School, Chicago, Ill., Public Health Service, U. S. Dept. of Health, Education and Welfare, Atlanta, Ga., and the N. Y. Medical College, New York City.

There is considerable suggestive evidence in the literature that the liver may be involved in the formation of serum complement and that the latter is diminished in liver disease and in experimental hepatic injury. Reports prior to 1929 were reviewed by Goldner(1). They indicate diminution of serum complement (C′) in experimental phosphorus or alcohol poisoning and its disappearance following hepatectomy in rabbits. In perfusion experiments with heat-inactivated serum, guinea pig liver contributed complement to perfusing serum, enabling the latter to hemolyze sheep red cells. On the other hand, complement remained normal in abdominally eviscerated dogs which had retained their livers. Goldner, using a rabbit hemolysin-sheep cell system in which *complete* hemolysis was used for the C′ titer, found that C′ was subnormal in 8 of 20 cases of "icterus catarrhalis" and in 10 of 11 cases of (cholangitic and portal) cirrhosis; furthermore, 3 of 7 cases of metastatic tumor of the liver showed diminution or absence of complement, one case of acute yellow atrophy complete disappearance of C′, while 6 cases of cholecystitis revealed no change from normal. Recently, Jordan(2), using his hematocrit method, reported marked diminution of C′ in all of 18 cases of cirrhosis studied; 22 cases of hepatitis showed some drop in C′ near the peak of the disease, while 12 patients with obstructive jaundice yielded high values. In contrast to these findings, Dulaney(3), using a 50%-hemolysis endpoint as determined pho-

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TABLE I. Serum Complement in 65 Patients with Hepatobiliary Disease.

Diagnosis	No. of patients	Age of patients, yr Range	Mean	Serum bilirubin, mg/100 ml Range	Mean	Serum complement, units Range	Mean
Acute hepatitis							
Virus A or B	16 (3)	13–60	30	1.1–31.2	14.7	1.4–5.7	3.2
Leptospiral	3	37–52	44	12.0–22.8	18.3	3.8–4.1	3.9
Toxic	1		22		38.8		1.2
Cirrhosis							
Alcoholic	18 (12)	25–56	40	.4–27.0	5.3	1.2–4.7	2.8
Posthepatitic	2	47, 75		3.5, 9.6		.8, .8	
Biliary obstruction							
Benign	9*	28–60	48	.8–28.0	9.9	2.0–6.1	4.0
Malignant	16†	39–72	57	.8–34.0	9.6	1.9–4.9	3.5

( ) denotes presence of complicating conditions.
* Acute (2 cases), chronic cholecystitis (4), common duct stone (3).
† Carcinoma of gastrointestinal tract (4 cases), pancreas (9), biliary tract (2) and kidney (1), causing jaundice and/or metastatic hepatomegaly.

tometrically, detected no significant changes in C′ titer in 25 patients with liver disease, including 5 cases of infectious hepatitis and 12 of cirrhosis. However, in the course of the same investigation, this author found C′ diminished in each of 24 patients with induced malaria.

In an attempt to clarify this apparent conflict in the literature, the present study was undertaken.

Materials and methods. Collection of all blood specimens was done in Atlanta† from subjects in pre-prandial state. Only those patients were included whose diagnoses appeared established either by autopsy, biopsy, surgical operation, or prolonged clinical observation. Twenty healthy persons served as normal controls, 65 patients had various forms of liver or biliary tract diseases (Table I), and 25 patients had miscellaneous illnesses. These included pneumonia (3 cases), acute rheumatic fever (5), infectious mononucleosis (1), aseptic meningitis (1), chronic pancreatitis (3), rheumatoid arthritis (1), sickle cell anemia (2), sarcoidosis (1), lymphoma (1), multiple myeloma (1), myocardial infarct (1), malignant hypertension (1), disseminated lupus (1) and acute (1) and burned out stage of glomerulonephritis (2). Promptly upon separation from the clots, the sera were stored at –40°C and, packed with dry ice, were transported by air to New York for analysis (K.L.), by the method previously reported(4). Fifty % hemolysis was used as endpoint. A serum which produced 50% hemolysis in the same dilution as a standard 1:8 guinea pig serum was said to contain 1 unit of C′. Obviously then, a serum which produced 50% hemolysis at twice the dilution of the standard serum contained 2 units, etc. In our experience the simultaneous testing of a standard guinea pig serum in each test represents an indispensable control over many variables, especially the changing fragility of the sheep cells.

Results. The serum complement ranged in the normal control group from 1.5 to 4.4 units, with a mean of 2.7. Corresponding figures for the abnormal control group, exclusive of one case of acute glomerulonephritis and one of disseminated lupus, were 1.4-4.7, and 2.9. The C′ titer of the latter 2 cases was severely diminished to 0.1 and 0.5 unit, respectively. In previous studies involving 166 control cases, one of the authors (K.L.) had found a normal range of 1.1 to 3 units with an average of 1.7(5). Hence, for classification of the present results, 1.1 units was taken as the lower limit of normal and 1.1 to 1.4 units were considered as borderline range. Two distinctly subnormal values in the hepatobiliary disease group (Table I) were obtained in the 2 patients with posthepatitic cirrhosis, 1 and 2 weeks, respec-

† Grady Memorial Hospital, Emory University Hospital and Vet. Adm. Hospital.

tively, before their deaths from hepatic insufficiency. Three other cases of uncomplicated portal cirrhosis with ascites and low serum proteins had borderline titers. C′ titers in all other cases tabulated were either within or above the normal range. While these values represent initial examinations upon admission to the hospital, the majority of these patients had one or more follow-up determinations; in no instance was a subnormal value encountered.

Discussion. It is a well accepted fact that inflammation may result in a rise of C′ (4,6,10-13). This fact seems to be borne out by the cases of benign obstructive jaundice, many of whom had an accompanying cholecystitis and who showed the highest mean C′ values, as well as by the majority of the hepatitis patients, who showed elevated C′ values. From the data presented, it appears that hepatocellular disease does not lower the C′ titer. The slight diminution of C′ in the 2 cases of posthepatitic cirrhosis should probably be rather attributed to the moribund state of these patients than to the presence of hepatic disease. A drop in C′ associated with extremely grave conditions, including overwhelming infections has been observed by other authors(1,3,7).

The discrepancy between our data and those of Goldner(1) and of Jordan(2) cannot be readily resolved, but may be justifiably attributed to differences in analytical technics. Certainly, the method used in this study could be trusted to reveal evidence of subnormal C′ levels, which it has uncovered with great consistency in acute and subacute glomerulonephritis, in nephrosis and in disseminated lupus erythematosus(4,5).

The observed absence of a C′ drop in hepatitis would suggest that there is no widespread antigen-antibody reaction taking place in this disease, in contrast to glomerular diseases and to hypersensitivity states, in which such a reaction has been postulated(4,5,6-9).

The lack of a C′ diminution in hepatocellular disease speaks against formation of C′—or, at least, its limiting component—in or through the liver‡ and tends to shift attention to the reticulo-endothelial system as its physiologic source. In contradistinction to Jordan(2), who speaks of "the great discriminatory power of the complement test," our data do not indicate that this offers any aid in the differential diagnosis of liver disease, with the exception, perhaps, of a grave prognosis in the occasional event of C′ diminution.

Summary. The serum complement titer was determined in 20 patients with acute hepatitis, 20 with hepatic cirrhosis, and 25 with (benign and malignant) biliary obstruction. The only distinctly subnormal values were encountered in 2 moribund patients suffering from cirrhosis. The data speak against formation of complement in or through the liver and fail to indicate usefulness of complement titration in diagnosis of hepatobiliary diseases.

‡ Authors found C′ elevated in 2 patients in whom about two-thirds of the liver had been surgically removed because of hepatoma.

1. Goldner, M., *Dtsch. Med. Wchschr.*, 1929, v55, 390.

2. Jordan, F. L. J., *Acta Med. Scandinav.*, 1953, v144, 268.

3. Dulaney, A. D., *J. Clin. Invest.*, 1948, v27, 320.

4. Lange, K., Graig, F., Oberman, J., Slobody, L., Ogur, G., and LoCasto, F., *Arch. Int. Med.*, 1951, v88, 433.

5. Lange, K., Slobody, L., and Strang, R., *Pediatrics*, 1955, v15, 156.

6. Wedgewood, R. J. P., and Janeway, C. A., *ibid*, 1953, v11, 569.

7. Fischel, E. E., and Gajdusek, D. C., *Am. J. Med.*, 1952, v12, 190.

8. Kellett, C. E., *Lancet*, 1952, v2, 911.

9. Stavitsky, A. B., *J. Imm.*, 1952, v69, 63.

10. Hadjopoulos, L. G., *J. Lab. and Clin. Med.*, 1928, v14, 131.

11. Ecker, E. E., Seifter, S., Dozois, T. F., and Barr, L., *J. Clin. Invest.*, 1946, v25, 800.

12. Fischel, E. E., Pauli, Ruth H., and Lesh, J., *ibid.*, 1949, v28, 1172.

13. Vaughan, J. H., Bayles, T. B., and Favour, C. B., *Am. J. Med. Sc.*, 1951, v222, 186.

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