STUDIES ON ALBUMINOCHOLIA

I. A SEROLOGICAL METHOD FOR DETERMINATION OF PROTEIN IN THE HUMAN BILES

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There has been no agreement concerning the excretion of coagulable protein into the bile in diseased conditions. As the coexistence of mucin and mucin-like substances in the bile prevents the separation of coagulable protein, no useful methods for the determination of bile protein have as yet been developed.

As a close relationship, as has been pointed out by Matsuo [1] and Mizuta [2], exists between the functions of the kidney and those of the liver, the detection of protein in the bile seems no less important than that in the urine. The previous methods employed for this purpose are to examine grossly the presence of coagulable protein by boiling the bile after treating mucin with acetic acid (Brauer [3], 1901; Raue [4], 1927). Aonther method newly deviced by Henning and Fischer [5] (1949) is a nonchemical one, in which a droplet of bile of a certain diameter is dried and the microscopic size of the protein ring is measured to determine the amount of protein. These methods, however, are not accurate.

In 1930, Matsuda [6] employed a serological method, by which he verified the appearance of autogenous protein in the bile of rabbits in the case of liver damage. Though his method seemed to be of some value, there has been no further application of this method to the hnman bile since the publication of his work. As hinted by his method, the present authors have attempted to prove serologically the so-called albuminocholia (Biossin) in the human bile collected by the Lyon tube, and have so far been obtaining nearly satisfactory results. In this report, our method and some data obtained therewith are presented.

METHODS

1. Preparation of immune serum: A dose of 1.0 ml human serum, which was freshly separated and diluted by physiological saline solution up to 5 ml, was given intravenously to several rabbits at intervals of three or four days for five times. Seven days after the last dose, a small amount of blood was removed from an ear-vein, and the separated serum was tested for precipitin. If the serum was positive at 1 : 5000 dilution (reported here as 5000× in terms of dilution), the animal was bled totally, the serum was inactivated at 56°C. for 30 min., and kept in the refrigerator, after phenol being added to the rate of 0.5 per cent.

2. Estimation of precipitin titer: The immune serum thus prepared was placed undiluted in Uhlenhuth tubes to the height of 5 mm with a capillary pipette, and the serial dilution (1:10, 1:20, 1:40, ·····) of human serum, the protein concentration of which had been made previously at 5 gm/dl, are overlayed gently. The readings were taken after one hour in the incubator at 37°C. The precipitin titer obtained ranged from 10,000× to 130,000×, and the minimal amount of protein to be proved by these antisera is theoretically 0.038 to 0.5 mg/dl, assuming the protein used as an antigen to be monovalent.

3. Bile: The clear parts of the bile, which was obtained by duodenal sounding using a Lyon tube and confirmed to be unmixed with gastric juice, were used for test. The gall-baldder bile was aspirated by infusion of magnesium sulphate solution. Each of the three fractions of bile was not always tested.

4. Procedures of determination: The bile was diluted by the normal saline 1:1, 1:2, 1:4, ····· preferably until 1:1024, and the diluted bile was overlayed on the undiluted immune serum in a series of Uhlenhuth tubes. The determination of the precipitin was carried out in the same way as described for the estimation of precipitin titer. Special care was required to make clear the contact lines between the two liquids. This procedure must be finished as soon as possible after aspiration. The readings were made at one hour in the incubator, and after being kept at room temperature overnight.

5. Reading and interpretation: The reaction was recorded as ###, ##, +, ⊥, ±, —, according to the degree of the white rings formed around the contact lines, and the reading was made by the dilution of the last positive tube or the tube of (⊥) in the same way as in recording the precipitin titer. In the tubes of less diluted bile, the white rings were sometimes widened and looked doubled. This phenomenon may be attributed to the polyvalency of the antigen.

The correlation between the time and the reaction is shown in Table I. The reaction proceeded for a few hours in the incubator. However, because of possible occurrence of turbidity caused by coexisting germs, it is recommended to make the readings in one hour. Not infrequently, the reaction was seen in one or three more tubes the following day.

Results

Various patients were examined by the mentioned procedures, and for the sake of the interpretation of data, they were divided into three groups.

Group I. Diseases of the liver (Table II): Thirteen cases of hepatic diseases, comprising seven cases of infectious hepatitis, two cases of cirrhosis of the liver, and one case of chronic hepatitis, hepatosplenomegaly, hypertrophic cirrhosis of the liver and distomatosis of the liver respectively, were included. The results, which are shown in Table II, indicate that every test revealed a reading from 4× to 64×, with an exception of Case 15.

TABLE I

Correlation between the time and the reaction

Bile	Time of incubation (min.)	Dilution of the bile									
		1	2	4	8	16	32	64	128	256	510 *
A	30	++	+	+	±	−	−	−	−	−	−
	60	++	+	+	+	−	−	−	−	−	−
	90	++	+	+	+	±	−	−	−	−	−
	120	++	+	+	+	+	±	−	−	−	−
	150	++	+	+	+	+	±	−	−	−	−
C	30	++	+	+	+	±	−	−	−	−	−
	60	++	+	+	+	+	±	−	−	−	−
	90	++	+	+	+	+	+	±	−	−	−
	120	++	+	+	+	+	+	±	−	−	−
	150	++	++	+	+	+	+	±	−	−	−
B	30	++	+	+	+	±	−	−	−	−	−
	60	++	++	+	+	+	±	−	−	−	−
	90	++	++	+	+	+	+	+	−	−	−
	120	++	++	++	+	+	+	+	±	−	−
	† over night	++	++	++	++	+	+	+	+	+	±

* The number indicates the degree of dilution, i.e., 4 means 1 : 4 and 8 means 1 : 8 dilution of the bile.

† Kept at room temperature.

TABLE II

Diseases of the liver (Group I)

Case	Name	Sex	Age	Disease	Bile	Dilution of the bile										Precipitin titer of the antiprotein-serum
						1	2	4	8	16	32	64	128	256	512	
1	M. N.	♂	14	inf. Hepatitis	A	+	+	+	±	−	−	−	−	−	−	10240
2	Y. S.	♂	58	Hepatic cancer	A	++	++	+	+	+	+	+	±	−	−	〃
4	S. Y.	♂	15	inf. Hepatitis	A	++	+	+	±	−	−	−	−	−	−	〃
					C	++	+	+	+	−	−	−	−	−	−	〃
5	H. M.	♂	61	inf. Hepatitis	A	++	++	+	+	−	−	−	−	−	−	〃
					C	++	++	+	+	−	−	−	−	−	−	〃

TABLE II Continued

Case	Name	Sex	Age	Disease	Bile	Diultion of the bile										Precipitin titer of the antiprotein-serum
						1	2	4	8	16	32	64	128	256	512	
12	S. K.	♀	45	Hepatosple-nomegaly	A	+	+	+	±	−	−	−	−	−	−	10240
					B	+	+	+	±	−	−	−	−	−	−	〃
15	H. N.	♂	19	inf. Hepatitis	A	+	±	−	−	−	−	−	−	−	−	〃
					B	+	−	−	−	−	−	−	−	−	−	〃
17	N. A.	♂	22	inf. Hepatitis	A	++	+	+	+	−	−	−	−	−	−	〃
					B	++	+	+	+	+	−	−	−	−	−	〃
18	A. H.	♀	17	inf. Hepatitis	A	++	++	++	+	+	+	±	−	−	−	〃
					B	++	++	++	+	+	+	±	−	−	−	〃
21	Y. T.	♂	52	cirrhosis of the liver	A	+	+	+	±	−	−	−	−	−	−	20480
22	K. S.	♀	21	distoma-tosis of the liver	A	++	+	+	+	−	−	−	−	−	−	81920
					B	++	+	+	+	±	−	−	−	−	−	〃
					C	++	+	+	+	+	±	−	−	−	−	10240
23	S. M.	♂	25	chr. Hepatitis	B	++	++	+	+	−	−	−	−	−	−	81920
24	K. N.	♂	34	cirrhosis of the liver	B	++	++	+	+	−	−	−	−	−	−	〃
30	S. T.	♂	46	Hypertor-phic cirrhosis of the liver	A	++	++	+	+	−	−	−	−	−	−	〃
					B	++	++	++	+	+	±	−	−	−	−	〃

Group II. Diseases of the bile ducts (Table III): This group comprised, cholelithiasis, 11; diskinesia of the gall-bladder, 2; tumor of the gall-bladder, 1; and trichomonas infection 1; totalling 15 cases. The readings ranged between 2× and 256×, except for A-bile of Case 9, and the majority of them were 8× or thereabout.

Group III. The other diseases (Table IV): Two cases of nephrosis, one case of gastric cancar, kidney tuberculosis and pancreatitis respectively, were taken for control. All but the case of pancreatitis - alternatively cholelithiasis - were below 4× in reading, and Case 10 and 11 did scarcely react in undiluted tubes.

OTHER FACTORS INFLUENCING THE REACTION

1. Trypsin: It was tested if trypsin, being possibly admixed in the bile, could alter the amount of detectable protein by digestion. Inactivation of trypsin was made by acidifying the bile with hydrochloric acid to pH 6–6.5. The reading was compared with the untreated bile (Table V). No significant difference could be seen between the two materials.

TABEL III

Diseases of the bile ducts (Group II)

Case	Name	Sex	Age	Disease	Bile	Dilution of the bile											Precipitin titer of the antiprotein-serum
						1	2	4	8	16	32	64	128	256	512	1024	
3	H. U.	♀	44	Cholelithiasis	A	+	+	+	−	−	−	−	−	−	−	−	10240
					B	++	++	+	+	+	+	−	−	−	−	−	〃
6	K. N.	♂	42	Cholelithiasis	A	++	+	+	+	+	±	−	−	−	−	−	〃
					C	++	+	+	+	+	+	±	−	−	−	−	〃
7	H. K.	♂	48	Cholelithiasis	A	++	+	+	±	−	−	−	−	−	−	−	〃
					B	++	+	+	±	−	−	−	−	−	−	−	〃
					C	++	+	+	±	−	−	−	−	−	−	−	〃
8	H. D.	♂	24	Diskinesia of the gall-bladder	A	+	+	+	+	−	−	−	−	−	−	−	〃
					B	++	++	++	++	+	±	−	−	−	−	−	〃
					C	+	+	±	−	−	−	−	−	−	−	−	〃
9	I. O.	♀	44	Cholelithiasis	A	+	−	−	−	−	−	−	−	−	−	−	〃
					B	++	+	+	±	−	−	−	−	−	−	−	〃
14	K. K.	♂	36	Cholelithiasis	A	++	++	+	+	±	−	−	−	−	−	−	〃
					B	++	+	+	+	+	±	−	−	−	−	−	〃
19	H. S.	♂	64	Cholelithiasis	A	+	+	+	+	±	−	−	−	−	−	−	〃
20	S. M.	♂	28	Cholelithiasis	C	++	++	++	++	+	+	+	+	+	±	−	25600
25	Y. M.	♂	29	G-bladder tumor	B	++	++	+	+	+	−	−	−	−	−	−	〃
26	S. A.	♀	66	Cholelithiasis	B	++	+	+	+	−	−	−	−	−	−	−	10240
27	S. F.	♂	39	Cholelithiasis	A	++	++	++	+	−	−	−	−	−	−	−	〃
					B	++	++	++	+	−	−	−	−	−	−	−	〃
29	Y. F.	♂	22	Cholelithiasis	B	+	+	−	−	−	−	−	−	−	−	−	81920
31	T. H.	♂	27	Cholelithiasis	B	++	++	++	+	−	−	−	−	−	−	−	〃
32	M. K.	♂	34	Trichomonas infection	A	+	+	+	+	−	−	−	−	−	−	−	〃
					B	++	++	++	++	++	+	+	+	+	−	−	〃
33	K. S.	♀	22	Diskinesia of the gall-bladder	A	+	+	+	+	−	−	−	−	−	−	−	〃
					B	++	++	++	+	+	+	−	−	−	−	−	〃

TABLE IV

The other diseases - control (Group III)

Case	Name	Sex	Age	Disease	Bile	Dilution of the bile									Precipitin titer of the antiprotein-serum
						1	2	4	8	16	32	64	128	256	
10	S. F.	♂	32	Nephrosis	A	±	−	−	−	−	−	−	−	−	10240
					B	+	−	−	−	−	−	−	−	−	〃
11	K. M.	♂	54	Gastric cancer	B*	±	−	−	−	−	−	−	−	−	〃
13	S. N.	♂	46	Pancreatitis @ (or cholelithiasis)	A	##	+	+	+	+	±	−	−	−	〃
					B	##	##	##	+	−	−	−	−	−	〃
					C	##	##	+	+	−	−	−	−	−	〃
16	M. T.	♀	22	Tuberculosis of the kidney	A	+	+	−	−	−	−	−	−	−	〃
28	Y. K.	♂	29	Nephrosis	C	+	+	+	±	−	−	−	−	−	81920

* Aspirated directly from the gall-bladder during operation.
@ Diagnosis was not established.

TABLE V

Influence of trypsin on the reactions. Comparison of untreated bile and acidified bile

Bile	pH	Dilution								
		1	2	4	8	16	32	64	128	256
A	Untreated	##	+	+	+	−	−	−	−	−
	Acidified	##	+	+	+	−	−	−	−	−
B	Untreated	##	##	+	+	+	±	−	−	−
	Acidified	##	##	+	+	+	−	−	−	−
C	Untreated	##	+	+	±	−	−	−	−	−
	Acidified	##	+	+	+	−	−	−	−	−

2. A tranparent and colorless layer appearing at the contact line: A phenomenon, though not frequent, that a thin, lucid and colorless layer appears in some cases at the lower part of the bile in tubes close on the boundary, was studied. The reading, however, was not disturbed by this phenomenon, as the reaction occurred beyond this layer. This study was made by using the biles aspirated from the gall-bladders during surgical operations. The same phenomenon was reproduced, which may as well exclude the possibility that the layer is formed either by gastric or pancreatic juice.

DISCUSSION

Anti-protein rabbit sera were employed for the determination of protein in the human bile. It is apparent that the degree of precipitation differs roughly in proportion to the amount of protein, and the reading of precipitation can be interpreted to represent the amount of protein to be estimated. As far as the immune serum contains a known and satisfactorily high precipitin titer, it seems justified to claim our serological method to be semiquantitative. A considerably wide range of the readings for albuminocholia in different diseases, and the distinct differences between the results in the diseases either of the liver or the bile ducts and those in the control, substantiate this view.

The weakly positive reaction in the control cases, though not enough regarding the number of cases, should be considered to be due to the high sensitiveness of the anti-protein sera employed. The same phenomenon has also been reported in the normal human urine.

Inasmuch as the immune serum was not produced by sensitization with highly purified fractions of protein, it will not be allowed to regard the precipitation as resulting exclusively from coagulable protein. However, it is evident that the coagulable protein constitutes the greatest part of the human serum protein when analysed quantitatively. As far as the precipitin test retains its value for medicolegal and other purposes to detect the human serum, and as far as the antigenic properties of the serum protein are taken into account, the precipitation produced in our procedures can be considered as showing the presence of serum protein.

And, it will be reasonable to understand that the degree gf precipitation presents almost exactly the degree of albuminocholia.

Conclusions

1. A new serological method, which is based upon the protein-antiprotein system, for the determination of albuminocholia is described. Using this method, the amount of protein excretion in the human bile was estimated.

2. This method is useful to determine semiquantitatively the coagulable protein in the bile, and the reaction employed is quite sensitive.

3. As applied to a number of patients, this method yielded varying degrees of albuminocholia, the maximal value being 1:256 dilution of the bile.

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