

STUDIES ON ALBUMINOCHOLIA
II. THE INFLUENCE OF MUCOPROTEIN ON THE
DETERMINATION OF ALBUMINOCHOLIA

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Little is known about "albuminocholia", a condition of bile which is comparable to "albuminuria" of urine. For determination of "albuminocholia" or coagulable protein in bile, a precipitin reaction was employed and described in a previous communication, together with a discussion about the clinical significance of this condition.

In a previous report (1) it was demonstrated that bile obtained from some of diseased patients showed a marked precipitin reaction with immune sera, while bile obtained from a control groups produced only a slight precipitin reaction. Inasmuch as bile contains a certain amount of mucin and mucin-like substances, the interference of mucoprotein present in serum antigens on the specificity of immune sera was further investigated. This report deals with the interference of mucoprotein separated from human serum and bile on the determination of "albuminocholia".

EXPERIMENTAL METHODS

The preparation of immune sera and the precipitin reaction with bile have been described in a previous report (1). For the preparation of anti mucoprotein sera the same procedure was followed except for the use of approximately 1 mg. of mucoprotein per injection which was equivalent to the mucoprotein separated from 4 ml. of pooled human serum.

The method of Winzler et al. (2) was employed for the separation and purification of serum mucoprotein. Serum was diluted with distilled water one to two, and the same amount of 1.8 M perchloric acid as serum was added to this dilution. The mixture was then centrifuged so that the supernatant was decanted 5 minutes after the addition of acid. The supernatant was dialyzed, and precipitated at pH 4 with saturated ammonium sulfate. The precipitate, then, was redialyzed, and the dialysate was tested with Nessler's reagent to assure complete dialysis. After being condensed to the original serum volume, the dialysate was employed for sensitization.

In order to obtain a separation of bile mucoprotein from coagulable bile protein, it was necessary to modify the method of Winzler et al. The bile was diluted and treated with acid in the same way as serum. However, it was not possible to centrifuge to get rid of precipitated material so that it was necessary to use a Seitz filtration instead. The filtrate was dialyzed, reprecipitated with alcohol at 85 per cent, washed with absolute alcohol several times, dried, dissolved in distilled water, precipitated with saturated ammonium sulfate, dialyzed and quantitatively condensed to the original tyrosine content of bile protein. The tyrosine

content of bile protein was used as standard to determine the amount of mucoprotein to be used for the reaction.

Bile protein was prepared as follows. The bile was precipitated with alcohol at 85 per cent, and the precipitate washed with alcohol and acetone repeatedly until the washing did not give any phenol color reaction. The precipitate was washed with ether, dried, dissolved in 1/5 saturated sodium carbonate, hydrolyzed and the tyrosine content determined. Folin-Ciocalteu's method (3) was used for the determination of tyrosine.

A saliva mucin which was used as mucoprotein control was prepared in the following way. To two volumes of saliva were added 8 volumes of absolute alcohol; the precipitate was filtered, washed with absolute alcohol and ether alternately for several times, and dissolved in the same amount of water as the original volume of saliva.

EXPERIMENTAL RESULTS

The bile of 12 specimens from 8 different patients were examined for their precipitin reaction with (a) anti human serum mucoprotein rabbit serum (anti M. P.) and (b) anti human whole serum rabbit serum (anti W. S.). The results of these reactions are given in Table I. The data show that bile precipitin reac-

TABLE I
Precipitin reactions given by anti serum mucoprotein rabbit serum

Case	Specimen Bile*	Anti human whole serum rabbit serum (undiluted)									Anti mucoprotein rabbit serum (undiluted)						
		Dilution of bile									Precipitin titer	Dilution of bile					Precipitin titer against human serum
		1	2	4	8	16	32	64	128	256		1	2	4	8	16	
1	B	++	+	+	+	+	-	-	-	-	20480 ×	+	+	±	-	-	2 ×
2	A	+	+	+	+	-	-	-	-	-	∅	+	±	-	-	-	∅
3	A	++	+	+	+	-	-	-	-	-	∅	+	±	-	-	-	∅
	B	++	+	+	+	+	±	-	-	-	∅	+	+	+	-	-	∅
4	B	++	++	++	+	+	+	-	-	-	∅	++	+	-	-	-	∅
5	C	+	+	+	+	-	-	-	-	-	∅	+	±	-	-	-	∅
6	A	++	++	+	+	+	+	-	-	-	∅	±	-	-	-	-	∅
	B	++	++	++	+	+	+	-	-	-	∅	+	+	+	±	-	∅
	C	++	+	+	+	+	±	-	-	-	∅	+	-	-	-	-	∅
7	B	++	++	++	+	+	+	+	±	-	∅	+	+	+	-	-	∅
8	A	++	+	+	+	+	±	-	-	-	∅	+	±	-	-	-	∅
	B	++	++	+	+	+	+	+	±	-	∅	+	+	-	-	-	∅
Urine		+	+	-	-	-	-	-	-	-	∅	±	-	-	-	-	∅
Salive mucin		+	±	-	-	-	-	-	-	-	∅	+	-	-	-	-	∅

* A, B, C indicate fractions of bile

tions with anti W.S. were of the order of 1 : 8 to 1 : 128 while its reaction with anti M.P. was only 1 : 2 to 1 : 4. Urine and saliva mucoprotein produced no appreciable precipitin reactions with either anti W.S. or anti M.P.

In order to determine the nature of the material reacting with anti M.P. serum, two procedures were used, (a) a precipitin reaction with bile mucoprotein and (b) the reaction given by the filtrate of heated bile.

The results of the precipitin reaction between human bile mucoprotein and undiluted anti W.S. and anti M.P. sera are given in Table II. No reaction greater than 1:1 was observed in any of these experiments. On the other hand, the reaction of the mucoprotein present in a filtrate of heated bile gave the same precipitin reaction as untreated bile with anti M.P. serum. This filtrate was prepared by first acidifying bile with N/20 HCl. Acid is added to the extent that one or two more drops would produce turbidity, pH usually being 6.0 to 6.5. The bile was then heated at 100°C for 30 minutes and filtered. The presence of mucoprotein is indicated by the precipitation produced if acetic acid is added. This filtrate was tested for precipitin reaction with anti W.S. and anti M.P. sera, and the titers were compared with those of the original bile that was not treated.

TABLE II
Precipitin reactions of bile mucoprotein

Anti serum	Bile	Dilution of bile mucoprotein					Precipitin titer
		1	2	3	4	8	
Anti human whole serum rabbit serum	A	±	-	-	-	-	2048 ×
	B	+	-	-	-	-	
	C	±	-	-	-	-	
Anti human serum mucoprotein rabbit serum	A	+	-	-	-	-	2 ×
	B	+	-	-	-	-	
	C	±	-	-	-	-	

As shown in Table III. the precipitation with anti W.S. was much less in the heated filtrate than in the unheated bile, and the titers given by the filtrate was of the same order of magnitude as those obtained by the reaction between anti M.P. and the original bile. It may be noted that bile mucoprotein prepared by perchloric acid precipitation did not give as much reaction as that prepared by heat treatment. It was presumed that perchloric acid precipitated a fraction of the mucoprotein reacting with anti M.P.

TABLE III
Precipitin reactions of bile before and after heat treatment.

Antisera	Dilution of bile (before treatment)								Dilution of filtrate (after treatment)					Bile fraction
	1	2	4	8	16	32	64	128	1	2	4	8	16	
Anti whole serum rabbit serum	†	†	+	+	+	+	-	-	+	+	+	-	-	A
	†	†	†	+	+	+	+	-	+	+	±	-	-	B
Anti mucoprotein rabbit serum	+	+	+	-	-	-	-	-	+	+	±	-	-	A
	†	+	+	-	-	-	-	-	+	+	+	-	-	B

DISCUSSION

The results obtained in Table I indicate that mucoprotein in human serum produce antibodies in the rabbit during sensitization using human whole serum and this anti human serum mucoprotein precipitin interferes with the original reaction between human bile and anti human serum rabbit sera. However, the interference was less than four fold. Therefore, bile revealing titers higher than 1:8 or 1:16 by original method should be considered as representing "albuminoholia", and those titers divided by the titers given by anti M.P. shown roughly the real precipitin titers given by coagulable protein in bile. The data in Table III also support this assumption in that the reduction in titer after elimination of most of the coagulable protein by heat treatment of the bile was almost the same as the difference between the titers of the original bile given by anti W.S. and by anti M.P.

From the practical point of view, our original procedures are still useful in evaluating the degree of albuminoholia, since anti M.P. sera produce only slight reactions.

SUMMARY

It was demonstrated in this report that mucoprotein from human serum and bile interferes only to a slight degree with the precipitin reaction between human bile and anti human whole serum rabbit sera (anti W.S.). This does not seriously affect our original procedures described in a previous report for detecting the presence of albuminoholia, that is, the coagulable protein in the bile. A correction may be applied with anti human serum mucoprotein rabbit sera (anti M.P.) for more precise determinations.

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