

The findings described above, except the presence in the bile for 24 hours of appreciable amounts of free and combined sulfanilamide, are illustrated in the results of these experiments upon one subject shown in the table. As already stated, these results are essentially similar to studies made upon 4 other subjects. The experiment presented also shows that when a second dose of sulfanilamide was ingested at a time when the drainage from the biliary tract contained the drug in both the free and the combined forms, the increase in the amount of the total compound was largely or wholly in the free state. In the urine on the other hand, the larger proportion of the increase was the combined (acetyl) derivative.

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Occurrence of Precipitation Zones in Mixtures of Serum and Sodium Desoxycholate; Significance in Pneumococcolysis.*

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Observations by Ransom,¹ Bayer,² Sellards,³ Ponder,⁴ and Williams⁵ have shown that the lysis of red cells by bile salts is inhibited in the presence of serum. Wieland,⁶ and Donnelly and Mitchell⁷ have subjected this fixation of bile salts by serum to quantitative study. The latter felt that the bile salt-serum reaction, which manifested the Danysz effect, was one of adsorption rather

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¹ Ransom, F., *Deutsche Med. Wochenschr.*, 1901, **27**, 194.

² Bayer, G., (a) *Biochem. Z.*, 1907, **5**, 368; (b) *idem.*, 1908, **9**, 58.

³ Sellards, A. W., *J. H. Hosp. Bull.*, 1908, **19**, 268.

⁴ Ponder, E., *Proc. Roy. Soc., B.*, 1922, **93**, 86.

⁵ Williams, J. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **29**, 916, 918.

⁶ Wieland, H., *Naunyn-Schmiedeberg, Arch. Pharm. und exp. Path.*, 1920, **86**, 79, 92.

⁷ Donnelly, J. L., and Mitchell, A. G., *Am. J. Physiol.*, 1927, **79**, 297.

TABLE I.
Lysis of an 18-hour Culture of Pneumococcus III.
1 ml of 4% serum broth culture plus 1 ml of various concentrations of sodium desoxycholate, in plain broth.
Final Dilution of Sodium Desoxycholate.

37°C	1:250	1:500	1:750	1:1,000	1:1,250	1:1,500	1:2,500	1:5,000	1:10,000	Broth Control
Serum	Immed.	T+	T+	T++	T++	T++	T++	T++	T++	T++
Broth	30'	C	T+	T+	T+	T+	T+	T+	T+	T+
Culture	60'	C	T+++	T+++	T++	T+	T+	T+	T+	T+

Key to all tables:

- C = Clear.
- T+, T++, T+++ = Various degrees of turbidity.
- ff = Fine flocculation.
- cf = Coarser flocculation.
- Immed. = Immediately.
- OV = Over night.

than of chemical combination. They demonstrated that the reaction quantitatively followed the adsorption equation, $\frac{X^n}{C} = K$. This, however, according to Sobotka,⁸ is not appreciably different from the earlier mass action formulation of Wieland, $\frac{aB}{(A-a)^2} = K$. Carl-
infante,⁹ has recently reported the occurrence of turbidity in mixtures of 5% human serum and diluted ox bile. In his experience, turbidity did not occur with dilutions of bile greater than 1 to 40.

During the course of experiments dealing with the quantitative determination of pneumococcal capsular polysaccharide in lysates, we noted certain irregularities in the clearing of 4% serum broth cultures (Pn. III) by solutions of sodium desoxycholate. Portions of this culture had been diluted 1 to 2 with broth containing the desired concentration of sodium desoxycholate, so that the final mixture contained horse serum in a 2% concentration. Under these conditions cultures containing concentrations of desoxycholate ranging between approximately 1 and 2 mg per ml failed to clear after several hours at 37° C and overnight refrigeration while with several higher and lower concentrations the same cultures cleared completely in one half hour at 37° C. (Table I). Smears and cultures from the turbid fluids containing 1 to 2 mg per ml of desoxycholate, however, failed to reveal bacteria.

The reactions were, therefore, repeated with plain as well as serum broth cultures. The middle zone of turbidity did not develop with plain broth cultures nor did control tubes of plain broth, without organisms, become turbid in the presence of those concentrations of desoxycholate employed in the lysis studies. The pneumococci grown in serum-broth were washed 3 times in saline and one-half the sediment resuspended in plain saline and the other half in 4% serum saline. These were also diluted 1 to 2 with broth containing various concentrations of sodium desoxycholate. The plain saline suspension cleared completely with concentrations of sodium desoxycholate up to and including 1-2500; the serum saline suspension became turbid in the same zone encountered with the unwashed serum broth cultures. Control tubes with a final concentration of 2% serum became turbid in the identical zone. (Table II).

We employed the technique of Donnelly and Mitchell,⁷ mixing varying concentrations of sodium desoxycholate with 2% horse

⁸ Sobotka, H., *Physiological Chemistry of the Bile*, Williams and Wilkins, Baltimore, 1937, page 131.

⁹ Carlinfante, E., *Sperimentale, Arch. di Biol.*, 1938, **92**, 8.

TABLE II.
Lysis of 18-hour Plain Broth Culture of Pneumococcus III and of Washed Serum Broth Culture Resuspended in Saline with or without Serum.
(Final concentration of serum—2%.)
Final Dilution of Sodium Desoxycholate.

37°C	1:250	1:500	1:750	1:1,000	1:1,250	1:1,500	1:2,500	1:5,000	1:10,000	Diluent
Plain broth*	Immed.	T+	T+	T+	T++	T++	T++	T++	T++	T++
	30'	C	C	C	C	C	C	T+	T+	T+
	OV	C	C	C	C	C	C	T+	T+	T+
Washed sediment suspension in saline	Immed.	T++	T+	T+	T+	T+	T++	T++	T++	T++
	30'	C	C	C	C	C	T+	T+	T+	T+
	OV	C	C	C	C	C	T+	T+	T+	T+
Washed sediment suspension in 4% serum saline	Immed.	C	T+	T+	T+	T+	T++	T++	T++	T++
	30'	C	T+	T+	T+	T+	T++	T++	T++	T++
	OV	C	T++	T++	T++	T++	T++	T++	T++	T++
4% serum broth without organisms	Immed.	C	C	C	C	C	C	C	C	C
	30'	C	T++	T++	T++	T++	T++	T++	T++	T++
	OV	C	T++	T++	T++	T++	T++	T++	T++	T++

*The density of this culture was adjusted to equal that of the 18-hour culture described in Table I (determined by photometer).

TABLE III.
 Development of Turbidity and Flocculation in Mixtures of Sodium Desoxycholate and Normal Horse Serum. Final Concentration of Horse Serum 2% in All Tubes.
 Final Concentration Sodium Desoxycholate.
 Mg per ml.

At room temperature	4.0 (1:250)	3.4	3.0	2.4	2.0 (1:500)	1.6	1.2	1.0 (1:1000)	0.6	Controls	
										0 (2% serum)	1.6 (No serum)
5'	C	C	C	T+	T+	T+	C	C	C	C	C
10'	C	C	C	T+	T+	T+	C	C	C	C	C
15'	C	C	C	T+	T+	T+	C	C	C	C	C
30'	C	C	C	T+	T+	T+	T+	C	C	C	C
60'	C	C	C	T+	T+	T+	T+	C	C	C	C
90'	C	C	C	T+	T+	T+	T+	C	C	C	C
120'	C	C	C	T+	T+	T+	T+	C	C	C	C
OV	C	C	C	T+	T+	T+	T+	C	C	C	C
					ff	ef	ff				

Powdered sodium desoxycholate, C.P., was dissolved directly in plain broth for use in experiments recorded in Tables I and II. The salt was evaporated from a 1% alcoholic solution before use in the experiment of Table III.

serum broth to a constant volume of 5 ml.[§] Table III shows the development of turbidity and flocculation in these mixtures and in the control tubes. Turbid fluids were not cleared by centrifugation at 2,000 rpm for 20 minutes nor by boiling. Addition of 3% acetic acid precipitated the bile salt. Normal NaOH in excess cleared the turbid mixture.

It will be noted in Table III that a 2% horse serum broth remained clear in the presence of 4 mg per ml of sodium desoxycholate while a tube containing 2 mg per ml became turbid promptly and had developed fine flocculation after standing overnight. To 5 ml of the turbid solution containing 2 mg per ml of the bile salt an additional 10 mg of dry sodium desoxycholate was added. There was prompt and complete clearing of the solution. The addition of an excess of serum to another turbid fluid failed to clear the mixture. The pH of the solutions containing 2 and 4 mg per ml of sodium desoxycholate, respectively, was determined with the quinhydrone electrode and both were found to be at 7.3. An alteration of pH was apparently not the factor responsible for the clearing.

We have also observed that solutions of sodium desoxycholate of greater than 2.0 mg per ml concentration, either in broth or in phosphate buffer near neutrality, gelled after standing at room temperature. This gelation occurs in the absence of serum and, as was also noted by Schaub and Reid,¹⁰ disappears on warming. Preliminary observations apparently indicate that gelation is more rapid and complete at neutrality than it is on the alkaline side of neutrality. The occurrence of this gel in cultures containing bile salts at a pH of 6.8 has been previously described by Mair¹¹ and is an evidence of their colloidal character of which others have been described.^{4, 12-14}

The present observations indicate that serum interferes with the interpretation of lysis in the presence of certain concentrations of sodium desoxycholate. However, several text-books offer con-

§ The reactions were unaffected when a Sørensen phosphate buffer at a pH of 7.3 was substituted for peptone broth as diluent. Stock powdered sodium desoxycholate did not dissolve in broth until heated to 65°C. After evaporation from alcohol, however, rapid solution took place at room temperature. The pH of the mixture of serum with the highest concentration of desoxycholate employed was 7.3, determined with the quinhydrone electrode.

¹⁰ Schaub, I. G., and Reid, R. D., *J. A. M. A.*, 1938, **111**, 1285.

¹¹ Mair, W., in *A System of Bacteriology*, Med. Res. Council, 1929, London, Vol. II, page 168.

¹² Achard, C., Boutaric, A., and Berthier, P., *Compt. Rend. Acad. Sci. (Paris)*, 1937, **204**, 1049.

¹³ Bashour, J. T., and Bauman, L., *J. Biol. Chem.*, 1937, **121**, 1.

¹⁴ Roepke, R. R., and Mason, H. L., *J. Biol. Chem.*, 1940, **133**, 103.

flicting opinions regarding the influence of serum on the lysis of pneumococci by bile salts.^{11, 15, 16}

Summary. Turbidity and flocculation developed in 2% horse serum broth containing concentrations of sodium desoxycholate between 1.2 and 2.4 mg per ml at a pH of 7.3 and cleared on the addition of an excess of sodium desoxycholate. Apparent failures of lysis of serum broth cultures of pneumococci were due to the occurrence of such turbidity because no bacteria were found in these mixtures.

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Influence of Arsenicals, Bismuth and Iron on the Plasma Ascorbic Acid Level.

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Several reports indicate that the poisonous effects of a number of drugs like benzene, lead,¹ phenylcinchoninic acid,² and glycerol³ and especially the arsenicals¹ may be counteracted successfully by giving suitable doses of ascorbic acid.

From examination of the urine Dainow⁴ concluded that patients who showed symptoms of intolerance to arsenicals were in a state of hypovitaminosis C. By administration of ascorbic acid, these hypersensitive patients became able to tolerate neoarsphenamine. Other investigators⁵⁻¹¹ reporting similar observations, emphasize

¹⁵ Boecker, E., and Kauffmann, F., *Bakteriologische Diagnostik*, 1st Ed., 1931, Berlin, J. Springer, p. 71.

¹⁶ Park, W. H., and Williams, A., *Pathogenic Microorganisms*, 10th Ed., 1933, Lea and Febiger, Phila., p. 353.

¹ Abt, A. F., and Farmer, Chester J., *The Vitamins*, Chapter XXII, American Medical Association, Chicago, 1939.

² Bertellotti, L., *Minerva, Medic.*, 1939, **30**, 254.

³ Pfeiffer, C., and Arnove, T., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **37**, 467.

⁴ Dainow, T., *Presse méd.*, 1937, **45**, 1670; *Annal. Dermat. et Syphil.*, 1939, **10**, 139.

⁵ Landfisch, S., *Polska gas. lek.*, 1937, **16**, 575; *J. A. M. A.*, 1937, **109**, 834.

⁶ Cormia, F. E., *Canad. Med. Assn. J.*, 1937, **36**, 392.

⁷ Montesano quoted by Bertellotti.²

⁸ Biss quoted by Dainow.⁴

⁹ Tibor, S., *Orvosi Hetilap*, 1939, **83**, 811.

¹⁰ Santiago, A., *Zbl. Haut und Geschl. Krkh.*, 1938, **60**, 74.

¹¹ Takahashi quoted by Bertellotti.²