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# Free and Combined Sulfanilamide in Material Drained from the Human Biliary Tract.

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The concentrations of free and of combined (presumably acetyl) sulfanilamide have been determined in the material collected through T-tubes inserted in the bile ducts of human subjects after operations upon the biliary system. Bile was clarified by a mixture of trichloracetic and phosphotungstic acids. Free sulfanilamide was determined by the method of Marshall and Litchfield[1] after the addition of acetone to the filtrate from the pigments etc. removed by this procedure. In the determination of combined sulfanilamide, the filtrate was treated with hydrochloric acid and hydrolized for an hour in a boiling water bath before the diazo reaction was carried out. Both free and acetyl sulfanilamide* were added to the bile and were recovered quantitatively by this procedure, but the method of precipitating could not be considered as wholly satisfactory, for a trace of pigment (apparently biliverdin) was present after treatment in a fair number of specimens from some of the patients studied. The method of Marshall and Litchfield was also used in the study of urine specimens collected simultaneously with the bile from a number of the patients. Blood analyses were carried out by the technique of Bratton and Marshall.[2]

Although the clarification of some of the bile specimens was not satisfactory the results of 10 studies upon 5 patients agreed closely together, and justify the following statements. There was no compound in the clarified bile which gave a reaction with the diazo technique used. Shortly after one to 2 g of sulfanilamide were given by mouth, the free drug appeared in the bile draining from the T-tube, but regularly in a concentration lower than that present in the blood. This finding differs from results previously reported upon human bile from the gall bladder[3] for the concentration of free sulfanilamide in such material is frequently higher than it is

1 Marshall, E. K., Jr., and Litchfield, J. T., *Science*, 1938, **88**, 85.

* The acetyl sulfanilamide used was a synthetic product furnished through the courtesy of the National Aniline and Chemical Company.

2 Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

3 Bettman, R., and Spier, E., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 463.

TABLE I.
Concentration of Sulfanilamide in Urine, Blood and Drainage from the Biliary Tract.

| Period 4 hr each | Fluid drained from biliary tract | | | | | | Blood | | Urine | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Apr. 6 | | Apr. 11 | | Apr. 14 | | Apr. 11 Apr. 14 | | Apr. 14 | |
| | Concentration of sulfanilamide—mg per 100 cc. | | | | | | | | | |
| | Free | Total | Free | Total | Free | Total | Free | Total | Free | Total |
| Before | — | — | 0.00 | 0.00 | — | — | | | — | — |
| | 2 g (xxx grains) of sulfanilamide given by mouth. | | | | | | | | | |
| 1st after | 0.76 | 0.70 | 1.60 | 1.66 | 1.77 | 1.75 | 2.87<br>2.25 | 3.70*<br>4.70† | 14.3 | 23.6 |
| 2nd " | 1.35 | 1.77 | 1.72 | 2.11 | 0.40 | 0.42‡ | | | 30.8 | 45.2 |
| 3rd " | 1.46 | 1.68 | — | — | 1.45 | 1.84 | | | 27.6 | 29.7 |
| | 2 g (xxx grains) of sulfanilamide given by mouth. | | | | | | | | | |
| 4th " | — | — | — | — | 1.58 | 1.74 | 1.80 | 2.50* | 20.8 | 37.7 |
| 5th " | — | — | — | — | 1.92 | 2.54 | | | 19.0 | 29.9 |
| 6th " | — | — | — | — | 2.37 | 2.96 | | | 18.1 | 22.3 |
| 7th " | — | — | — | — | 2.56 | 3.20 | | | 26.6 | 32.3 |

*Blood collected April 11, 1940.
†Blood collected April 14, 1940.
‡About 5 cc of very thin bile drained from the tube during this 4-hour period.
The time of the 4-hour periods of collection (before, 1st after, etc.) is calculated from time when the first dose of sulfanilamide was administered.
The blood specimens were collected at the mid-points of the 4-hour periods against which they are recorded.

in the blood. Later, the free sulfanilamide in our experiments approximated more nearly the concentration in the blood, as would be expected from the water content of the two fluids[4] but the relationship was not directly proportioned to the water content. Sulfanilamide could be demonstrated in the bile 24 hours after 2 g had been taken by mouth.

During the first 4 hours after the administration of such single doses as were used in these experiments, no demonstrable amount of combined form of the drug could be demonstrated in the bile, although both free and combined forms were found in the blood during this period. After 4 hours, both compounds were present, although the concentrations of the combined forms were small, for only 10 to 20% of the total sulfanilamide occurred as the combined derivative at periods when analyses of blood and urine showed that approximately half of the total sulfanilamide reacted with the diazo reagents only after acid hydrolysis. If acetyl sulfanilamide is formed in the liver, as current experimental work indicates[5] it is apparently not discharged in large amounts directly into the bile.

[4] Marshall, E. K., and Cutting, W. C., *J. Am. Med. Assn.*, 1937, **108**, 953; *J. Pharm. Exp. Therap.*, 1937, **61**, 196.

[5] Harris, J. S., and Klein, J. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 781; Klein, J. R., and Harris, J. S., *J. Biol. Chem.*, 1938, **124**, 613.

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 Williams[5] have shown that the lysis of red cells by bile salts is inhibited in the presence of serum. Wieland,[6] and Donnelly and Mitchell[7] 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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† Littauer Fellow in Pneumonia Research.

‡ Dazian Fellow.

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.