

which is made up to volume. To make up the standard containing 0.2 mg. per cc., 10 cc. of this solution is made up to 100 cc. For the standard containing 0.4 mg. per cc., 20 cc. of the solution is made up to 100 cc.

As a rule no more than 4 unknowns are prepared and read at one time, since the color tends to fade rapidly.

If the color of the unknown solution does not match in the colorimeter within 5 mm. of reading of one of the standards, measured portions are removed from the 100 cc. flask and suitably diluted or concentrated. Since different samples of bile vary in their content of cholic acid, this procedure gives a high degree of flexibility to the method.

Cholic acid added to bile of known bile acid composition could be recovered to within 5% of its theoretical value.

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Differential Quantitative Analysis of Bile Acids in Bile and in Duodenal Drainage.

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By the combination of 3 different methods the analysis of bile for taurocholic acid, glycocholic acid, total conjugated bile acids, cholic acid, deoxycholic acid, total bile acids, and free bile acids has been achieved. It has also been possible to analyze duodenal drainage material for cholic acid, deoxycholic acid and total bile acids.

Methods. 1. The Cuny¹ modification of the Schmidt-Dart² method is used to determine the taurine and glycine nitrogen percentage. The first is multiplied by 33.87 and the second by 28.29 to give the bile acids conjugated with taurine and with glycine. The sum of these 2 figures gives the total bile acids that are conjugated. 2. Cholic acid is determined by a method outlined in the previous paper.³ 3. The total bile acids are estimated by an iron precipitation method based partly on principles devised by Harwood.⁴ After

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¹ Cuny, L., "Le dosage des sels biliaries dans la bile et le liquide duodenal," Paris, 1930.

² Schmidt, C. L. A., and Dart, A. E., *J. Biol. Chem.*, 1920, **45**, 415.

³ Doubilet, H., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 84.

⁴ Harwood, R. U., *J. Lab. and Clin. Med.*, 1934, **19**, 1003.

the bile acids have been extracted and made up to 100 cc. in a volumetric flask and the cholic acid determined, the amount of solution in the volumetric flask, estimated to contain between 40-60 mg. of bile acids, is measured out into a 150 cc. beaker. This calculation is derived from the known amount of cholic acid present in the volumetric flask. The bile acids of dog bile may be considered to be approximately 100% cholic acid, while those of human bile can be assumed to be 50% cholic acid. Thus if the volumetric flask is found to contain 100 mg. cholic acid derived from dog bile, 50 cc. of the solution is measured in the beaker. If the extract is derived from human bile, only 25 cc. is taken.

The solution in the beaker is evaporated down to 30 cc. and 10 cc. of 8N KOH is added. The beaker is covered with a watch-glass and hydrolysis is carried out on a boiling water bath for 6 hours.

After hydrolysis is completed, 5N H_2SO_4 is added until the solution becomes acid, *i. e.*, the point at which the white cloudiness due to precipitated bile acids becomes permanent. About 10 cc. of 2N K_2CO_3 is then added to neutralize the excess H_2SO_4 , and to dissolve the precipitated bile acids. The contents of the beaker are brought to dryness on a water bath. The beaker is dried for 20 minutes in an oven kept at 110°C . After trituration the contents are extracted 3 times with 50 cc. quantities of light boiling (35°C .) petrolic ether. The petrolic ether is boiled down each time to half its volume on an electric hot plate covered with asbestos. The petrolic ether is filtered through a small funnel holding No. 43 Whatman's filter paper.

The beaker is allowed to stand until the residual petrolic ether has evaporated and is then placed in the 110°C . oven for 15 minutes. Absolute alcohol (15 cc.) is then added and, after stirring, the alcohol is filtered into a 100 cc. beaker through the same funnel and filter paper that had been used previously. The contents of the beaker are extracted 3 times more with 15 cc. quantities of alcohol, brought to the boiling point on a hot plate. The alcoholic solution is evaporated to dryness on a steam bath, and the bile salts transferred with small quantities of water to a 15 cc. centrifuge tube, the total volume being about 9-10 cc. With a drop of phenol red solution as indicator, 2-3 drops of 5N H_2SO_4 are added to neutralize any K_2CO_3 that may have come through in the alcoholic filtrate. The centrifuge tube is then heated for 5 minutes in the boiling water bath to drive off CO_2 formed. Just sufficient NaOH is added to make the solution alkaline and to dissolve the precipitated bile acids. The contents, while still hot, are brought to a pH of about 6.9 by the addition of N/1 HCl drop by drop, the optimum point

being where the pink color, due to the phenol red, just disappears. The centrifuge tube is cooled under the water tap and about 4 cc. of a 4% FeCl_3 solution mixed with an equal amount of borax—boric acid buffer of pH 6.95, is added with a pipette. After standing for 15 minutes, the precipitated ferric bile salts are centrifuged and the supernatant fluid discarded. The precipitate is washed twice with $\text{N}/2 \text{Na}_2\text{SO}_4$ to remove the excess ferric chloride. About 10 cc. of $2\text{N K}_2\text{CO}_3$ is then added and the centrifuge tube heated for half an hour in the steam bath. After centrifuging again, the supernatant fluid, containing the bile salts is discarded and the precipitated ferric carbonate is washed once with dilute K_2CO_3 to remove any bile salts still present. The precipitate is then dissolved in about 10 cc. $\text{N}/1 \text{HCl}$ and after carefully adding 3 drops of superoxol the centrifuge tube is placed in the boiling water bath for a half hour. The ferric chloride solution thus formed is transferred to a 25 cc. volumetric flask, which is made up to volume with $\text{N}/1 \text{HCl}$. The amount of iron is then determined.

If, for example, the iron precipitated by the bile acids is found to be 100 mg. % and the cholic acid in the same sample 1%, then the iron precipitated by the deoxycholic acid present will be equivalent to $100 - 45.4 = 44.6$ mg. % Fe (since 1 mg. of cholic acid combines with 0.0454 mg. Fe). Since 1 mg. deoxycholic acid combines with 0.0474 mg. Fe, deoxycholic acid = $\frac{44.6}{0.0474} = 941$ mg. % — 0.941%. Adding the deoxycholic acid (0.941%) to the cholic acid (1%) gives the figure for the total bile acids (1.941%).

By this method cholic and deoxycholic acids added to bile of known bile acid composition can be recovered to within 7% of their theoretical value.

4. The free bile acids are determined by subtracting the conjugated bile acids from the total bile acids.

Some typical examples of differential bile acid analysis are shown in Table I.

TABLE I.

Source of Bile	Conjugated Bile Acids			Cholic Acid	Deoxycholic Acid	Total Bile Acids	Free Bile Acids
	with Taurine	with Glycine	Total				
Human G. B.	3.799%	5.062%	8.861%	4.255%	5.496%	9.751%	0.890%
Canine G.B.	11.109	0	11.109	10.416	3.572	13.988	2.879
Human Fistula	1.061	0.288	1.304	0.626	0.881	1.717	0.413
Duodenal Drainage "A" Bile	—	—	—	0.129	0.118	0.247	—