STUDIES IN THE METABOLISM OF THE BILE.

III. THE ENTEROHEPATIC CIRCULATION OF THE BILE ACIDS.*

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The enterohepatic circulation of the bile was first postulated by Schiff (21) in 1870. Since then the behavior of the various chief constituents of the bile has been intensely studied. Broun, McMaster, and Rous (3) and McMaster and Elman (15), who have summarized the earlier literature on this subject, affirm the enterohepatic circulation of bile pigment, but this has not been substantiated by Stadelmann (22), Hooper and Whipple (11), Whipple (25), or by Bollman, Sheard, and Mann (2). In fact the preponderance of evidence seems to be against the circulation of bilirubin. The intestinal origin and enterohepatic circulation of urobilin and urobilinogen, postulated by Müller (18) has been fully confirmed by Meyer-Betz (17), Wilbur and Addis (26), Fischler (5), Rous (19), McMaster and Elman (14), and others and is now generally accepted. That the output of cholesterol in the bile is intimately related to the intake in the food is likewise accepted (McNee (16) and McMaster (13)).

The experiments and claims of Schiff (21) in regard to the enterohepatic circulation of the bile acids have since been amply substantiated by the work of Stadelmann (22–24), Foster, Hooper, and Whipple (6), Whipple (25), Brugsch and Horsters (4), and others. Schiff gave bile orally to a dog with a biliary fistula and

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measured the changes in the total solids of the bile. Stadelmann attempted to measure the bile acids chemically but because of difficulties in the analytic method was forced to use 12 hour samples of bile. Foster, Hooper, and Whipple (6), by means of their improved method, were able to work with 2 hour samples of bile, an interval still too great for a satisfactory study of the time relations involved. Whereas the enterohepatic circulation of the bile acids is generally accepted, it should be noted that Hoppe-Seyler (12) was unable to isolate them from the blood of the portal vein.

We have been able to follow the biliary excretion of bile acids and bilirubin at half hour intervals (1, 9, 10). In consequence it has been possible to determine the curve of excretion of the bile acids after their oral administration and to compare it with the effect of intravenous injection. We have also been able, by means of our colorimetric method for the determination of the bile acids (1), to compare the changes in the blood of the portal and systemic circulations.

Methods of Experimentation.

Experiments were performed on a dog with a permanent biliary fistula prepared according to the method of Rous and McMaster (20). Samples of the hepatic bile were collected at half hour intervals. Usually two preliminary samples were collected to establish the normal rate of flow of the bile. A solution of bile salts was then given by stomach tube, in a dosage equivalent to 100 to 125 mg. of glycocholic acid for each kilo of body weight and the collection of bile at half hour intervals continued for a period of 6 hours.

Additional experiments were performed on dogs under amytal narcosis. The gallbladder was removed and a cannula placed in the common bile duct. The hepatic bile so obtained was collected to establish the normal rate of flow. Bile salts, in a dose of from 100 to 125 mg. for each kilo of body weight, were then injected into the second portion of the duodenum and the collection of bile continued at half hour intervals for a period of 6 hours.

Samples of blood were obtained from the portal vein by means of a long narrow glass cannula so inserted as not to obstruct the portal circulation. In some experiments the spleen was removed

and the cannula inserted through the splenic vein. In other experiments one of the veins from the transverse colon was used and the cannula pushed down through the superior mesenteric vein until it projected into the lumen of the portal vein. When the cannula was not in use for the withdrawal of blood, it was closed by a pipe-stem cleaner moistened with heparin solution. The injection of heparin was also found to be of advantage in preventing thrombosis within the portal vessels.

The changes observed in the blood and bile following the intravenous injection of bile salts have been reported by Greene and Snell (9). The two series of experiments have been made as nearly comparable as possible and so indicate in part the effect of the method of administration on the behavior of the bile acids within the body.

EXPERIMENTAL.

A series of ten animals was studied. The data obtained in two typical experiments are given in Tables I and II. The changes in these two experiments are characteristic and illustrative of the whole series. Control experiments on a fasting animal showed that following the establishment of a biliary fistula and the external drainage of the bile there is a gradual diminution in both the volume of bile and the output of bile acids.

The normal response to the oral administration of bile acids to a dog with a permanent biliary fistula is given in Table I. A slight increase in the flow of bile was observed within 30 minutes after a solution of bile salts was given through a stomach tube. The increase was progressive and the greatest outflow of bile occurred during the second and third collection periods. Thereafter the volume gradually decreased, the period of maximal choleresis lasting about 3 hours.

The effect of the oral administration of the bile acids on their excretion in the bile is best shown when the total quantity rather than the concentration is studied. There was little change during the first period of collection. Thereafter there was a striking increase which corresponded in part to the changes in the volume of the bile. The maximal excretion occurred during the second, third, fourth, and fifth periods. The excretion then fell to the level noted in the control experiments.

Similar results were obtained in the experiments carried out under amytal narcosis (Table II) although the changes were not so marked and the recovery of the injected bile acids was much less complete. This was to be expected as considerable operative manipulation was unavoidable in these experiments, and our experience has indicated that the excretion of bile by the liver was

TABLE I.

Changes in Blood and Bile of Animal with Permanent Biliary Fistula Following the Oral Administration of Bile Acids.

Weight of dog, 14.6 kilos.		Blood.	Bile.					Remarks.
		Bile acids, mg. per cent.	Volume, cc.	Bilirubin.		Bile acids.		
Time of collection.				Mg. per cent.	Total mg.	Per cent.	Total mg.	
Before.	After.	Jugular.						
1 hr.		7.8	5.0	19	0.95	2.63	131	Control period.
½ "		7.9	5.0	22	1.13	1.50	75	
	15 min.	7.4						Bile acids given, 1460 mg. Bile acids recovered, 1663 mg.
	30 "	8.3	7.5	28	2.12	1.00	75	
	45 "	8.6						
	1 hr.	8.0	15.5	16	2.52	3.36	520	
	1½ hrs.		11.0	14	1.48	4.40	485	
	2 "	7.2	6.8	15	1.00	4.00	272	
	2½ "		3.1	19	0.59	3.36	101	
	3 "	6.9	3.0	28	0.86	1.96	59	
	3½ "		3.0	37	1.10	1.26	38	
	4 "	7.7	3.3	42	1.40	0.86	38	
	4½ "		3.0	47	1.40	0.75	22	
	5 "	7.4	3.1	43	1.33	0.68	20	
	5½ "		3.1	49	1.52	0.75	23	
	6 "	7.4	2.6	56	1.45	0.75	20	

markedly affected by such manipulation, the anesthetic used, or by disturbances in the portal circulation.

Greene and Snell (9) reported that after the intravenous injection of bile salts, choleresis was most marked within 30 minutes, and the changes took place within the first three collection periods. Approximately the total quantity of injected bile acids could be

recovered in the bile within 2 hours. Comparison of our results (Fig. 1) with their Table VIII shows that while the general curve of the excretion of bile acids is similar in both series of experiments, yet when bile acids are given by mouth their appearance in the bile is delayed and the period of excretion is prolonged.

TABLE II.

Changes in Blood and Bile Following the Oral Administration of Bile Acids.

Weight of dog, 11.8 kilos.		Blood.		Bile.					Remarks.
Time of collection.		Bile acids, mg. per cent.		Volume, cc.	Bilirubin.		Bile acids.		
Before.	After.	Jugular.	Portal.		Mg. per cent.	Total mg.	Per cent.	Total mg.	
½ hr.		7.3	7.4	1.9	75	1.42	1.43	27	Control period.
	15 min.	8.0	11.8						Bile acids given, 1380 mg. Bile acids recovered, 1051 mg.
	30 "	7.9	9.6	2.5	78	1.95	2.15	54	
	45 "	8.7	8.8						
	1 hr.	8.5	9.2	2.6	76	1.98	2.94	76	
	1½ hrs.	8.0	10.5	2.8	79	2.21	3.70	104	
	2 "	8.5	12.7	2.7	85	2.29	2.98	80	
	2½ "			2.5	86	2.15	3.03	75	
	3 "	8.9	11.2	2.0	90	1.80	3.15	63	
	3½ "			2.8	65	1.82	3.68	103	
	4 "	8.7	10.6	4.5	91	4.10	3.27	147	
	4½ "			3.1	73	2.26	3.47	104	
	5 "	9.4	19.1						
	5½ "			3.1	54	1.68	4.17	130	
	6 "	10.7	16.6	3.2	60	1.92	3.60	115	

This lag is evidently related to the time necessary for the absorption of the bile acids from the intestine.

The intravenous injection of a solution of bile salts produces a varying degree of hemolysis which is indicated by a subsequent increase in the excretion of bilirubin in the bile. When the bile salts are administered orally hemolysis does not occur and the excretion of bilirubin is unchanged during the experiment (Table I).

When bile acids are given orally, they are almost wholly with-

out effect on the systemic circulation. The analytic values in the peripheral blood were unchanged throughout the course of the different experiments. This was true not only when moderate doses of bile salts were given but when as much as 3 gm. for each kilo of body weight was given. When massive doses such as this are given some bile acid probably enters the peripheral circulation; various authors have reported the passage of small amounts into the urine. The quantities, however, are too small to affect the general level in the blood. In the fasting animal a significant difference did not exist between the intensity of the Pettenkofer

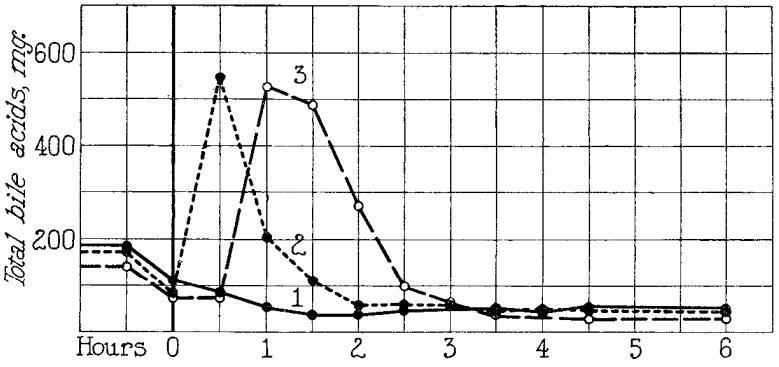

FIG. 1. Comparison of the changes in the excretion of bile acids in the bile in (1) control experiment and (2) after the intravenous administration of bile acids and (3) after the oral administration of bile acids.

reaction obtained in the arterial blood and that obtained from the jugular or portal veins.

An increase in the bile acid content of the blood from the portal vein was demonstrated within 15 minutes after the injection of a solution of bile salts into the duodenum (Fig. 2 and Table II). The analytic values were for the most part within a range of two to three times the normal. This increase in the bile acid content of the blood was not as marked as when an equivalent amount of bile acids was injected intravenously. On the other hand, the level in the blood was increased for a considerably longer time.

That this increase in the intensity of the Pettenkofer reaction obtained in the portal blood is due to the absorption of bile acids from the intestine is evidenced by Experiment 2, which shows an

accompanying increase in the flow of bile and in the output of bile acids during the period in which the level of bile acids in the blood from the portal vein was elevated. Considerable operative manipulation was unavoidable in these experiments. This not only had a definite effect on the excretion of the bile but when marked, as in the last periods of Experiment 2, permitted the passage of bile acids through the liver with a consequent terminal increase in the amount in the peripheral blood.

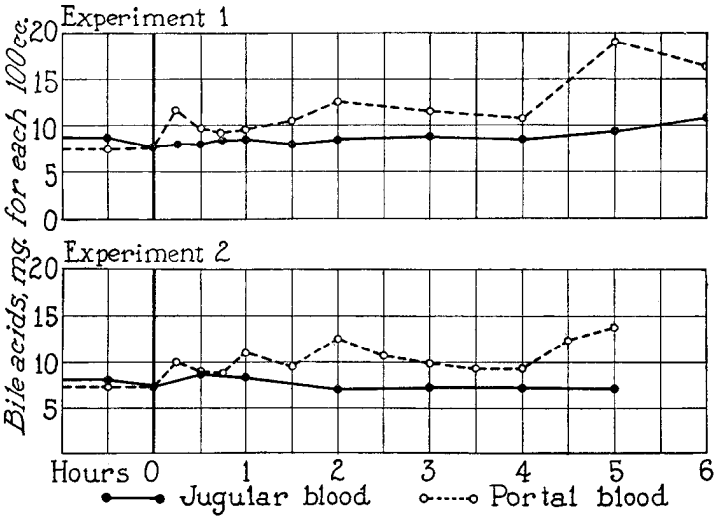

FIG. 2. The changes in the bile acid content of the blood from the jugular and portal veins following the oral administration of bile acids in Experiments 1 and 2.

We believe that under the conditions of the experiments these results can be accepted as evidence for the occurrence of bile acids in the blood from the portal vein during their absorption from the intestine. As such they form an additional link in the chain of evidence necessary to prove the presence of enterohepatic circulation of the bile acids.

SUMMARY.

Curves are presented to show the excretion of bile acids in the bile after their oral administration. In a dog with a biliary fistula there is a lag in the excretion as compared with the effect of intra-

venous injection that can be ascribed to absorption from the intestine.

The quantitative Pettenkofer test gives similar readings in the arterial, jugular, and portal blood of a fasting dog, but there is an increase in the amount of bile acids in the blood of the portal vein when a solution of the bile salts is injected into the duodenum. This confirms the earlier experiments of Schiff and others and extends our knowledge regarding the mechanism of the enterohepatic circulation of the bile acids.

BIBLIOGRAPHY.

1. Aldrich, M., and Bledsoe, M. S., *J. Biol. Chem.*, 1928, lxxvii, 519.
2. Bollman, J. L., Sheard, C., and Mann, F. C., *Am. J. Physiol.*, 1926, lxxviii, 658.
3. Broun, G. O., McMaster, P. D., and Rous, P., *J. Exp. Med.*, 1923, xxxvii, 699.
4. Brugsch, T., and Horsters, H., *Z. ges. exp. Med.*, 1923, xxxviii, 367.
5. Fischler, F., Physiologie und Pathologie der Leber nach ihrem heutigen Stande, Berlin, 2nd edition, 1925.
6. Foster, M. G., Hooper, C. W., and Whipple, G. H., *J. Biol. Chem.*, 1919, xxxviii, 379.
7. Greene, C. H., and Aldrich, M., *Am. J. Physiol.*, 1927, lxxxi, 480.
8. Greene, C. H., Aldrich, M., and Rowntree, L. G., *J. Physiol.*, 1927, lxiv, p. vii.
9. Greene, C. H., and Snell, A. M., *J. Biol. Chem.*, 1928, lxxviii, 691.
10. Greene, C. H., Snell, A. M., and Walters, W., *Arch. Int. Med.*, 1925, xxxvi, 248.
11. Hooper, C. W., and Whipple, G. H., *Am. J. Physiol.*, 1917, xlii, 264.
12. Hoppe-Seyler, F., *Arch. path. Anat. u. Physiol.*, 1863, xxvi, 519.
13. McMaster, P. D., *J. Exp. Med.*, 1924, xl, 25.
14. McMaster, P. D., and Elman, R., *J. Exp. Med.*, 1925, xli, 513.
15. McMaster, P. D., and Elman, R., *J. Exp. Med.*, 1925, xli, 719.
16. McNee, J. W., *Quart. J. Med.*, 1914, vii, 221.
17. Meyer-Betz, F., *Ergebn. inn. Med. u. Kinderheilk.*, 1913, xii, 733.
18. Müller, F., *Verhandl. Kong. inn. Med.*, 1892, xi, 118.
19. Rous, P., *Am. J. Med. Sc.*, 1925, clxx, 625.
20. Rous, P., and McMaster, P. D., *J. Exp. Med.*, 1923, xxxvii, 11.
21. Schiff, M., *Arch. ges. Physiol.*, 1870, iii, 598.
22. Stadelmann, E., Der Icterus und seine verschiedenen Formen. Nebst Beiträgen zur Physiologie und Pathologie der Gallensecretion, Stuttgart, 1891.
23. Stadelmann, E., *Deutsch. med. Woch.*, 1896, xxii, 785.
24. Stadelmann, E., *Z. Biol.*, 1897, xvi, 1.
25. Whipple, G. H., *Physiol. Rev.*, 1922, ii, 440.
26. Wilbur, R. L., and Addis, T., *Arch. Int. Med.*, 1914, xiii, 235.

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