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Bilirubin, Bromsulfalein, Bile Acids, Alkaline Phosphatase and Cholesterol of Thoracic Duct Lymph in Experimental Regurgitation Jaundice.

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Saunders[1] was the first to ligate the hepatic duct in dogs in 1795. Since then many others have attempted to elucidate the problem of regurgitation jaundice. An outstanding contribution and review on the subject is given by Mayo and Greene.[2]

The purpose of the present study was to determine the content of bilirubin, bile acids, alkaline phosphatase, and cholesterol, and of bromsulfalein following its administration intravenously, in the plasma and thoracic duct lymph of dogs, after ligation of the common bile duct.

Methods and Materials. Five male dogs were used ranging from 10 to 15 kg. Thirty to 40 mg of pentobarbital sodium per kg of body weight were used as anesthesia. The thoracic duct was cannulated at its entrance in the left jugular vein. Three drops of 25% sodium citrate solution were added to 10 cc of blood or lymph, to prevent coagulation, all determinations being made on plasma. Biliary obstruction was produced by a double ligation and severance of the common bile duct with previous ligation of the cystic duct. Bilirubin was determined by a modification[3,4] of Malloy and Evelyn's method.[5] Bromsulfalein was administered in the right external jugular vein in a dose of 2 mg per kg of body weight. The concentration of dye was then determined in the following manner:

Three cc of triethyl phosphate (Eastman Kodak Company) was added to 1 cc of plasma or lymph in a centrifuge tube. This was shaken thoroughly and centrifuged. Two cc of the supernatant fluid were then added to 8 cc of distilled water in an Evelyn tube. A blank reading was made in the Evelyn photoelectric colorimeter, using a 580 filter. Two drops of 20% KOH were then added to develop the color, and a reading was again taken, after which the concentration was de-

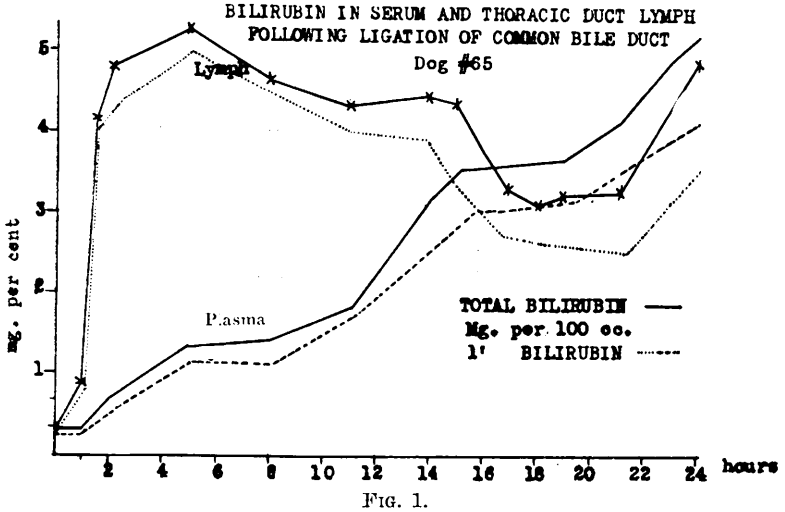

FIG. 1.

[1] Saunders, W. A., *A Treatise on the Structure, Economy, and Disease of the Liver*, William Phillips, London, 1809.

[2] Mayo, C., and Greene, C. H., *Am. J. Physiol.*, 1929, **89**, 280.

[3] Ducci, H., and Watson, C. J., *J. Lab. and Clin. Med.*, 1945, **30**, 293.

[4] Watson, C. J., *Blood*, 1946, **1**, 99.

[5] Malloy, H. T., and Evelyn, K. A., *J. Biol. Chem.*, 1937, **119**, 480.

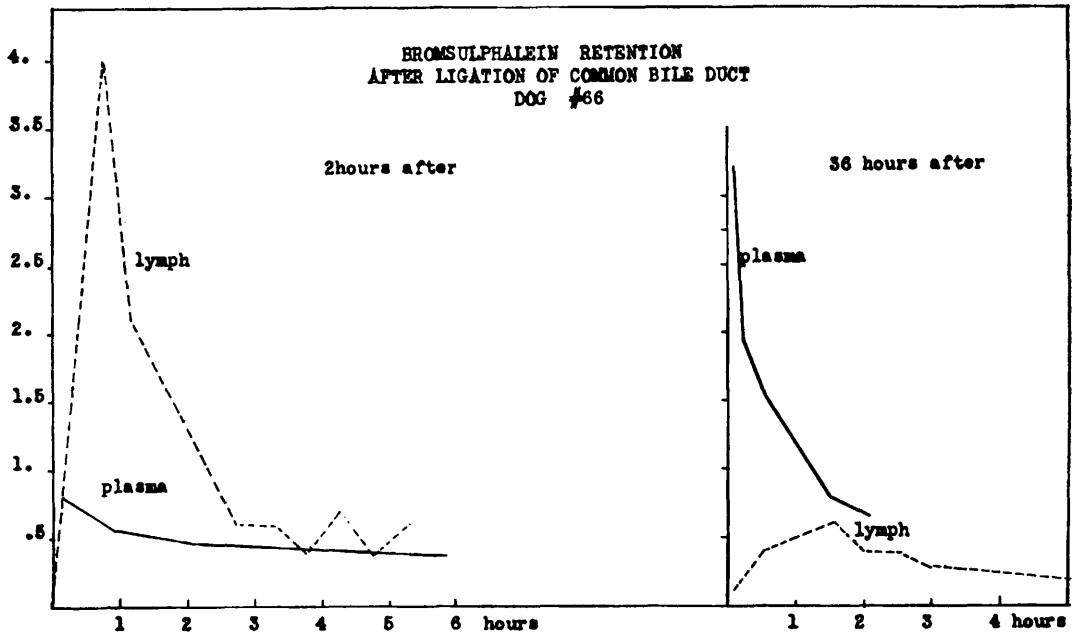

FIG. 2.

termined from a curve of the pure dye in known concentration. Bile acids were determined as cholic acid by Josephson's method.[6] Cholesterol was determined by the Sperry Schoenheimer method.[7] The alkaline phosphatase was determined by the method of King and Armstrong.[8]

Results. As seen in Fig. 1, bilirubin appears promptly in the thoracic duct lymph, being detectable in the blood a few hours later. Most of the bilirubin was of 1′ or prompt reacting type[3,4] in both lymph and plasma.

The data in Fig. 2 relates to injection of bromsulfalein at 2 hours and at 36 hours following the ligation of the common bile duct. In the early period of the obstruction, it is seen that the dye disappears promptly from the blood to be regurgitated at high concentration into the thoracic duct lymph. In the later period of obstruction, however, the dye is retained in the blood for several hours and little or none appears in the thoracic duct lymph.

Fig. 3 represents a typical experiment as regards bile acids in the thoracic duct lymph and blood, following ligation of the common bile duct.

These results are in accord with previous studies in which the Pettenkofer test was used, as discussed by Mayo and Greene.[2]

Typical data for the cholesterol in lymph and blood, in these experiments, is shown in Fig. 4. It is seen that the concentration of the total plasma cholesterol remained about the same during the 24 hours of observation. From this, it appears that if regurgitation of cholesterol from bile to lymph and thence to blood, does occur, it is considerably slower than that of the bile acids and the prompt reacting bilirubin.

Fig. 5 shows the rise in alkaline phosphatase in the blood and lymph. It is of considerable interest that distinct increases in phosphatase activity in thoracic duct lymph were observed.

Summary. The present results support the belief that, following common duct ligation, bile "regurgitates" into the lymph, and thence, via the thoracic duct into the blood. The appearance of bile acids and bilirubin in the thoracic duct lymph, as previously reported, is confirmed. The present study also reveals

[6] Josephson, B., *Biochem. J.*, 1935, **29**, 1519.

[7] Schoenheimer, R., and Sperry, W. H., *J. Biol. Chem.*, 1934, **106**, 745.

[8] King, E. J., and Armstrong, A. R., *Canad. Med. Assn. J.*, 1934, **31**, 376.

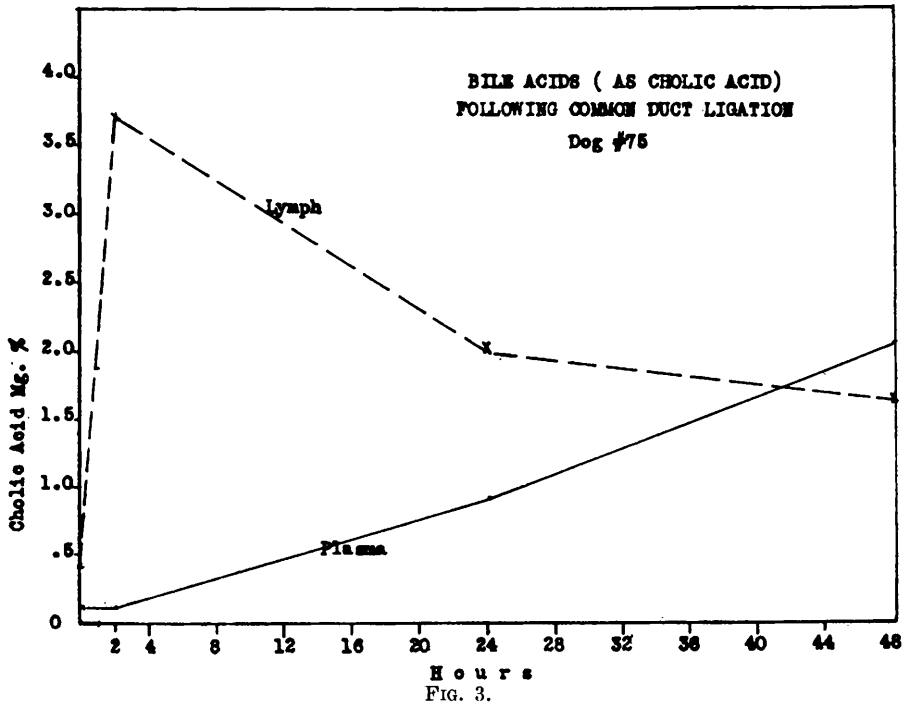

FIG. 3.

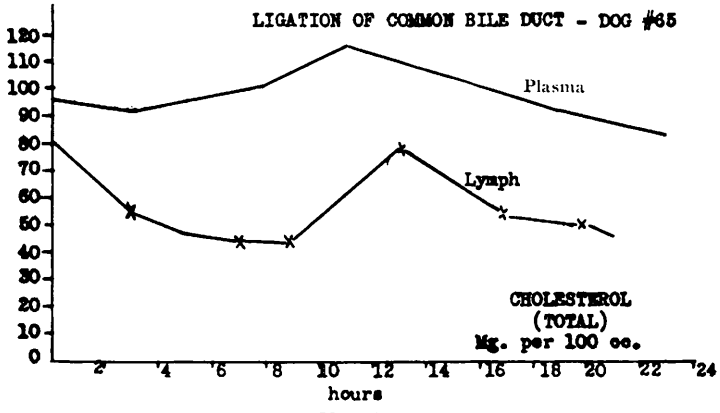

FIG. 4.

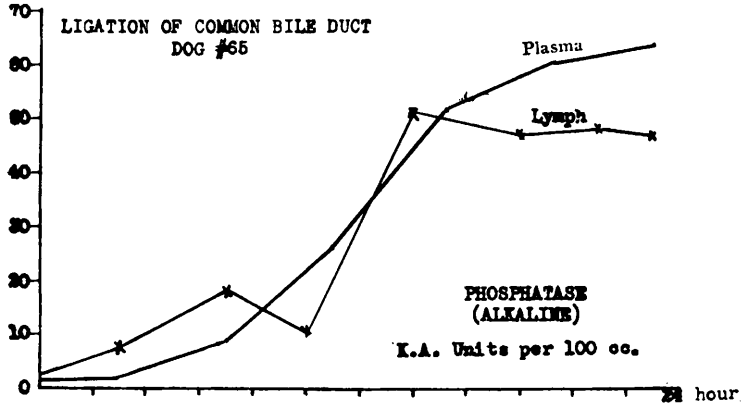

FIG. 5.

that the bilirubin of the lymph, under these circumstances, is mainly of the prompt reacting type, a finding in accord with the concept of a regurgitation of bile into the lymph.

During the early phase of biliary obstruction, the injected bromsulfalein quickly appeared in the lymph. After 36 hours, however, it was not removed from the blood and did not appear in the lymph in appreciable amount.

The cholesterol, on the contrary, did not increase significantly in the lymph within 24 hours, while the alkaline phosphatase behaved in an intermediary manner.

The writer wishes to acknowledge his indebtedness to Dr. C. J. Watson for helpful criticism and advice with relation to these studies.

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Effect of Anoxic Anoxia on Gastric Emptying Time of Rats Fed Corn Oil.

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Anoxic anoxia has been shown to cause a delay in the emptying time of the stomach in the dog and in man. Moreover, in man, as in the case of the dog, the more severe the degree of anoxia, the greater is the delay in gastric emptying.[1]

Studies on the effect of this type of anoxia on the absorption of fat from the alimentary tract in rats have shown that the amount of fat absorbed by animals subjected to partial pressures of oxygen of 63 mm and 53 mm Hg was significantly less than for control animals; the difference was greater for the greater degree of anoxia.[2] That these findings cannot be explained on the basis of a prolonged emptying time of the stomach, however, is shown by the following experimental results.

Using the technic previously described,[2] adult albino rats of both sexes, weighing between 200-300 g were given 1.5 cc corn oil (1.385 ± 0.01 g) and subjected to a partial pressure of oxygen of 53 mm Hg (7.03% oxygen) for 2-, 3- and 4-hour periods. Simultaneously-fed control animals were kept at atmospheric pressure. The amount of fat remaining in the stomach was quantitatively determined and calculated as per cent of the amount fed. Preliminary experiments on rats killed immediately after feeding showed that the mean percentage recovery of the amount of fat administered was 96.5 (range 92.3 to 99).

The data, Table I, show that the amount of

1 Van Liere, E. J., *Physiol. Rev.*, 1941, **21**, 307.

2 MacLachlan, P. L., and Thacker, C. W., *Am. J. Physiol.*, 1945, **143**, 391.

TABLE I.
Effect of Anoxic Anoxia on Gastric Emptying Time of Rats Fed Corn Oil.
Oxygen tension—53 mm Hg (7.03% oxygen).

Absorption time	2 hr		3 hr		4 hr	
	No. of rats	Fat in stomach* %	No. of rats	Fat in stomach %	No. of rats	Fat in stomach %
Controls	12	68.4	11	49.5	12	41.3
Anoxic	11	37.4	12	30.0	12	35.7
St. Dev.		17.0		16.5		13.4
P. (Fisher's)		<0.01		0.01		>0.3

* Expressed as per cent of the amount of fat fed.