STUDIES ON SERUM PHOSPHATASE ACTIVITY*

III. THE EFFECT OF COMPLETE BILIARY FISTULA ON PHOSPHATASE ACTIVITY IN SERUM AND BILE

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In Paper II of this series (1) experiments were reported on the increased phosphatase values of the serum produced by ligation of the common bile duct in dogs. The experiments demonstrated that the animal with complete biliary obstruction shows a steady rise of serum phosphatase which reaches a maximum about the end of the 2nd week.

It was found that ascorbic acid causes an activation of the serum phosphatase in vitro and that its addition to sera with high values obtained after common bile duct ligation also increases the phosphatase activity, but only to the same maximum obtained from the control sera before operation. It was concluded from these experiments that the observed rise in experimental obstructive jaundice was due to an increase in activity produced by some substance in the retained bile. It seemed of interest to study the phosphatase values in the serum of dogs with complete bile fistula in which the status of the bile would be just the opposite of that in experiments in which total retention of bile was produced.

Methods

The methods for the determination of serum phosphatase and the activation by ascorbic acid have previously been described in detail (2). The methods for phosphatase determination in the

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bile were as follows: Before the bile was used for phosphatase investigations, it was always centrifuged. It was evident from the start of the bile tests that the pigment in the bile would interfere with the colorimetric method used for phosphorus determination. This difficulty was overcome by adding petroleum ether to the samples after the cessation of enzymatic activity with 5 cc. of 10 per cent trichloroacetic acid, the bile pigments remaining in suspension, after shaking, in the petroleum ether layer. These were then removed with the precipitated proteins of the serum by filtration, a colorless filtrate resulting.

Complete biliary fistulae were established in a series of twelve dogs. All operative procedures were performed with complete aseptic technique and under full ether anesthesia. The abdomen was opened through a mid-line incision in each instance. The operative procedures were of two kinds. In some of the animals the common bile duct was ligated with black silk, a mushroom catheter inserted in the fundus of the gallbladder, and the opening in the gallbladder closed by a purse-string suture of silk. The end of the catheter was then brought out through a stab wound and attached by a short piece of glass tubing to a rubber balloon. In order to avoid the objection of possible contamination of the bile by various microorganisms, in some of the animals the technique described by Rous and McMasters (3) was used, in which a glass cannula is tied into the common bile duct and connected by rubber and glass tubing in such a way as to form a U of tubing inside the abdomen; this emerges through a stab wound and is also connected to a balloon.

Bile was collected quantitatively every 24 hours. The average survival time of the animals was 6 weeks. Blood samples were taken every 2 to 3 days in amounts not exceeding 15 cc. for each specimen.

In experiments on total bile fistula dogs, Enderlen, Thannhauser, and Jenke (4) and others found that several weeks after the bile fistula operation bone changes similar to osteoporosis are to be seen. After bile fistula, fat absorption is diminished and the stools contain large amounts of calcium soaps. The observed osteoporosis was explained by an increased calcium loss due to diminished absorption. It was of interest to study the phosphatase values in the serum in bile fistula where the experimental
conditions are just the opposite of those present in dogs with common bile duct ligation. One may assume that the blood phosphatase does not change under conditions in which the bile is totally drained through complete fistula.

The experiments (Table I) demonstrate the unexpected result that an increase of serum phosphatase is manifest also in bile fistula dogs. Ascorbic acid activates the serum of bile fistula dogs in the same way as in dogs with common bile duct ligation. Therefore, our assumption that an increased activity of serum phosphatase following common bile duct ligation may be applied in the same manner to the serum of bile fistula dogs, namely increased phosphatase values, must be explained by increased phosphatase activity.

The maximum increase was obtained on about the 7th day, after which there was some tendency toward a fall in the phos-

### Table I

**Serum and Bile Phosphatase after Bile Fistula Operation**

Temperature, 37°; pH 8.9, time of hydrolysis, 1 hour; amount of enzyme used, 0.1 cc.; substrate, sodium β-glycerophosphate.

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phatase values. The intensity of the activation of the serum with ascorbic acid, however, did not change as long as the dog lived. The fact that phosphatase activity increases immediately after the fistula operation fails to present evidence substantiating the former explanation that osteoporosis in these dogs is due only to a longer period of calcium loss.

The phosphatase values in the bile obtained from the fistula were consistently very high. In contrast to the serum, the phosphatase values in the bile could only be slightly increased by the addition of ascorbic acid. This experiment reveals the very important fact that the phosphatase is already present in the bile in a state of almost maximal activation.

DISCUSSION

The experiments gave the quite unexpected result of an increase of serum phosphatase in bile fistula dogs. The fact that an increase occurs seems to indicate that there are at least two factors in the bile; one which increases and another which decreases phosphatase activity. The factor in the bile which decreases phosphatase activity was found to be present in the bile acids, as will be reported in Paper IV following. The factor responsible for the increase of serum phosphatase activity seems to be some unknown substance which protects or influences the enzymatic system of the phosphatase.

According to Rothman, Meranze, and Meranze (5) high phosphatase values in the serum of jaundiced patients should be significant for obstructive jaundice. The finding of increased phosphatase values in bile fistula demonstrates that the diagnostic value of phosphatase determination in jaundiced patients is ambiguous from the point of view of the origin of the jaundice.

SUMMARY

1. Bile fistula dogs exhibit increased phosphatase values in serum after operation. The values are about 10 to 20 times as high as the initial value.

2. Ascorbic acid activates sera of bile fistula dogs to the same extent as it does the serum of dogs with common bile duct ligation.

3. In contrast to the increase of the phosphatase in the serum of dogs with biliary fistulae, the phosphatase in the bile itself
could not be activated to a great extent by ascorbic acid. The phosphatase in the bile already shows high phosphatase values before activation with ascorbic acid, so that the phosphatase must be present in a state of almost maximal activation. In differentiating obstructive jaundice from hepatocellular jaundice, the diagnostic value of serum phosphatase determination is not apparent.

4. The results indicate that the enzyme present in the serum which splits the β-glycerophosphate is a complex system, involving besides the enzyme and the substrate a substance which we may call the cofactor, producing high phosphatase activity.

BIBLIOGRAPHY

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