quite marked. On the other hand, in Dog B45 in which the greatest increase (300%) in the cholesterol content of the gallbladder bile was found, no inflammatory changes were made out. However, this experiment was of 16 days' duration, and an acute inflammation may have occurred and subsided, leaving no histological trace.

The high cholesterol content of the peritoneal fluid in Dog 830, where there was a localized peritonitis, may be explained by the fact that inflammatory exudates in general may contain much cholesterol.[1] The method for quantitating cholesterol was the colorimetric one and will be described in detail elsewhere. That used for bilirubin is described in a paper by Rous and McMaster.[2]

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Addition of Cholesterol to Hepatic Bile Subjected to Gallbladder Influence.

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To study the influence, if any, of the gallbladder on the cholesterol content of bile, a series of experiments were performed in which a "partitioning ligature" was so placed[1] that 2 portions of the liver were drained into separate rubber balloons through intubation of the common duct. One part of the bile flowed directly from the liver, whereas the other part was subjected to gallbladder influence, before flowing into the collecting bag. This device was first used by Rous and McMaster[1] in their classic studies on the concentrating activities of the gallbladder.

Dog. I, weight 12 kg., under ether anesthesia a ligature was placed around the common duct just proximal to 2 hepatic ducts which were then made to drain directly into a rubber balloon intubated at the termination of the choledochus. A second balloon was connected with the common duct proximal to the ligature. This drained the rest of the liver, including the gallbladder which, however, was first completely emptied. The abdomen was closed in layers and the bags left therein. Five days later the dog, in excellent condition, was killed with chloroform. The upper bag (in connection with

[1] Wells, H. G., Chemical Pathology, 5th Edition, Philadelphia, 1925, 301.

[2] Rous, P., and McMaster, P. E., *J. Exp. Med.*, 1921, **34**, 47.

[1] Rous, P., and McMaster, P. D., *J. Exp. Med.*, 1921, **34**, 47.

the gallbladder) contained 105 cc. of cloudy infected bile and was found to have drained 90 gm. of liver. The lower bag (from the liver directly) contained 205 cc. of clear bile and had drained 225 gm. of liver. Bilirubin comparisons to evaluate the degree of concentration by the gallbladder could not be accurately made because of the presence of infection in one of the specimens. Comparison of the cholesterol content revealed striking differences. The gallbladder bile contained 1.1 mg./cc., the hepatic bile but 0.06 mg./cc., values in accord with many estimations of isolated gallbladder and hepatic biles from many dogs, as will be reported later. The difference in the bile subjected to gallbladder influence is even more evident if one calculates the output per gm. of liver drained. The bile in the upper (gallbladder) bag contained 1.3 mg. of cholesterol per gm. of liver drained, the lower one but 0.05 mg. That concentration by the gallbladder could not account for these differences is obvious from the fact that 105 cc. and 225 cc. were put out by the 2 portions of the liver.

In 5 other dogs a simpler method of performing this experiment was adopted by using only one bag, emptying the gallbladder either by puncture through the wall (introducing the needle behind through the liver parenchyma to avoid the necessity of tying the puncture hole) or by passing a catheter into it through the opening in the common duct.

The output of bile pigment per gm. of liver drained was (with the exception of dog BB) almost exactly the same from each part of the liver. This agreement was taken as evidence that the bile flowing into the gallbladder and into the balloon from the 2 parts of the liver was primarily the same. Subsequent differences therefore could only be due to the action of the gallbladder on the bile flowing above the partitioning ligature. Since the experiment lasted but 48 hours the pressure in the isolated gallbladder system could not have become great enough to influence secretion.

The results confirm, as to the bile pigment content, the concentrating action of the gallbladder. The cholesterol of the gallbladder contents, however, was in each case greater than mere concentration of the bile could account for. This can be best seen by calculating the cholesterol output per gm. of liver drained. In the 5 dogs studied the gallbladder bile contained 153%, 122%, 27%, 18% and 67% *more* cholesterol per gm. of liver drained than the hepatic bile from the rest of the liver. These results indicate that in some way cholesterol was added to the bile by the gallbladder wall.

The rôle of infection seems to be an important one, first as shown by the first experiment described (Dog I) in which the infected gallbladder bile contained a very high content of cholesterol. In Dog Y, in which acute inflammatory changes were noted in the section of gallbladder wall, the greatest amount of the cholesterol was found in the gallbladder bile. Sufficient study of this factor, however, has not been made, although there is other evidence to be mentioned later which suggests also that infection may increase the output of cholesterol by the walls of the biliary tract.

Some of these dogs were fed large amounts of fat-containing food, but the effect of a high cholesterol diet is difficult to judge from these few experiments. There is, in general, much conflict of opinion as well as of evidence on this point.

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Cholesterol Content of "White Bile" from Various Sources, Including Contents of "Hydrops" of Gallbladder.

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During experiments on the biliary tract of dogs a number of instances of so-called white bile were observed, one from the gallbladder and the others from the ducts. Several human cases were also encountered in which hydrops of the gallbladder was found at operation. These specimens were all analyzed for their cholesterol content.

The significance of these observations concerns the question of the function of the gallbladder and bile ducts as regards the cholesterol of the bile. Is cholesterol absorbed or is it secreted by the bile duct and gallbladder epithelium? Or is it indifferent to the activities of these cells? Study of "white bile" furnishes one way of getting suggestive information as to these questions since it is well known that this secretion is a product not of the liver but of the duct mucosa. The colorless fluid found in hydrops of the gallbladder must similarly be a product of its wall since in these cases the cystic duct is occluded.

In 4 cases of hydrops in the human 2 gave 0.19, 0.45 mg. per cc. of cholesterol, and 2 cases gave macroscopic cholesterol crystals. Stepp and Nathan[1] found 0.64 mg./cc. of cholesterol in the contents