THE CHOLESTEROL FUNCTION OF THE GALL BLADDER

BY ROBERT ELMAN, M.D., AND J. B. TAUSSIG, M.D.

(From the Department of Surgery, Washington University School of Medicine, and Barnes Hospital, St. Louis.)

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Although cholesterol has achieved a widespread interest in relation to the general metabolism of the body it has an especial importance in surgery because of its striking tendency to localize and cause disease in the gall bladder. Gall stones nearly always contain cholesterol and many of them are composed of this substance alone. Even in the absence of stones it may be stored in large amounts under the mucous membrane, a not uncommon condition now called the "strawberry gall bladder," or more strictly "cholesterosis" of the gall bladder. Our attention has therefore been directed for many years to the behavior of this substance in the bile, especially as regards the activities of the gall bladder wall. Does the biliary epithelium excrete cholesterol in the bile, or does it absorb it? Although this question has been variously answered by writers on the subject, the problem still remains, it seems to us, an unsolved one. Evidence will be presented in this paper which, it is believed, helps to point to the true solution.

The name of Naunyn is associated with the early reports on cholesterol in relation to the gall bladder, although Virchow first suggested that the epithelium of the viscus had something to do with this fat-like alcohol. Naunyn, in 1892, first expressed the view that cholesterol in the bile is a product of the gall bladder and bile duct epithelium through desquamation and degeneration of their cholesterol-containing cells. This hypothesis was based on the study of much clinical material, on the accessory observation that inflammatory discharges from mucous membrane elsewhere (sputum) contained as much or more cholesterol than bile, and on the experiments of Jankau who found that cholesterol of the bile was independent of cholesterol intake.

Direct measurements of the cholesterol content of the bile from the gall bladder as compared with that from the liver might be expected to throw light on our problem. And many observations have been made, not only on autopsy material...
Wide variations were found in various cases. In the aggregate, however, a striking difference was generally observed; thus gall bladder bile contains much more cholesterol than hepatic bile. Fox places the average content of gall bladder bile (autopsy material) at 3.8 mg. per cc. and that of hepatic bile at 0.6 mg. per cc. This does not indicate necessarily however that the gall bladder must secrete cholesterol. The gall bladder mucosa desquamates rapidly after death, and the high values in gall bladder bile in autopsy material is partly due to cellular debris. Doyon and Dufourt found that by filtering gall bladder bile lower values of cholesterol are obtained. A second and more important objection is that the gall bladder concentrates bile and hence the higher cholesterol values may be merely due to inspissation. One can, of course, allow for this effect. Some analyses show that the cholesterol of gall bladder bile is much greater than can be accounted for by concentration (9), whereas others show the reverse (42). But most of the comparisons have involved gall bladder bile from one source and hepatic bile from another; and the variations from various sources are often tremendous. Doyon and Dufourt, it is true, found 0.25 mg. per cc. cholesterol in hepatic bile from a dog whose gall bladder bile contained 1.1 mg. per cc. They did not take into account however the concentrating influence of the gall bladder.

We come now to the opposite idea championed first by Aschoff and taken up by nearly all subsequent workers (7, 21, 25, 31), i.e., that cholesterol is absorbed from the bile by the wall of the gall bladder. The direct evidence favoring this view is rather limited. Torinoumi, a pupil of Aschoff's ligated the cystic duct in dogs, removed and measured the gall bladder contents replacing most of it, retaining only enough for analysis. In three dogs he found a decrease in the cholesterol content of the gall bladder bile after 1 to 4 weeks thus indicating absorption, although the decrease was not great. In two dogs, however, he found an increase, which he attributed to the fact that in them inflammatory changes in the gall bladder wall were present. The present authors in similar experiments (14, 17) found in only one of nine dogs any evidence of absorption and then it was quite insignificant. In all others there was a slight to a marked increase in the cholesterol of the gall bladder contents 2 to 16 days after ligation of the cystic duct. Inflammation was present in only one or two cases. Illingworth has described experiments on two cats with cystic duct occlusion. He used normal cat bile but mixed it with a large amount of cholesterol, perhaps 25 to 50 times its normal content, and after several days noted that a large part had disappeared. It is not improbable, however, that with an artificial bile containing so much cholesterol, most of it undoubtedly in suspension, the loss may have occurred through phagocytosis. Delrez and Cornet found that, when sterile mineral oil containing a known amount of cholesterol was placed in a dog's gall bladder after previously emptying it and ligating the cystic duct, an increased amount of cholesterol could be recovered after 2 days. Sections of the gall bladder showed no inflammation. We have repeated this experiment and found too a definite excretion of cholesterol in this way. In summarizing these experiments one must admit that they support the theory of excretion more than that of absorption.
Boyd found that rabbits, on a high cholesterol diet, following cholecystectomy showed a lower level of blood cholesterol. He assumed that the gall bladder's absorptive power had been removed. Sweet, on the other hand, found that the blood cholesterol of dogs rose to a higher level following a fat meal after the gall bladder was removed. Blaisdell and Chandler noticed a higher blood cholesterol in dogs with cystic duct occlusion. Hansen fed rabbits a high cholesterol diet and found cholesterol crystals in the gall bladder bile especially when the cystic duct was narrowed though he never found them in control unfed or unoperated animals. These indirect experiments would seem to indicate excretion rather than absorption.

There has been much microscopic study of cholesterol in the mucosa of the biliary tract. But one cannot tell in which direction microscopic fat is going, or whether indeed it is only evident as a result of trauma or other extraneous factors. Moreover the microscopic recognition of cholesterol may be extremely difficult. In general, therefore, microscopic studies would seem prima facie to be of relatively little value for the present problem, a view already expressed by others (4, 25). Nevertheless extensive study in several species by Shikinami showed no evidence indicating fat resorption by the bladder cells but instead that the cells produce a "mucoid" product. Aschoff on the other hand after introducing a variety of fats in the dog gall bladder after ligation of the cystic duct found them present in the mucosa and hence claimed that the gall bladder could absorb fat. It is significant however that cholesterol alone among the lipoids used has never been shown to be thus absorbed, though of course it has been repeatedly tried (3, 25, 26, 31). Indeed Aschoff suggested that perhaps only the cholesterol ester is absorbed, split, and the cholesterol part immediately excreted. While this hypothesis offers a compromise, no evidence to support it has ever been reported. Another compromise has been advanced by Kusnetzowsky who reports two kinds of cells in the mucosa of the biliary tract; one a large cylindrical cell which contained fat only after filling the ducts with oil and hence assumed to be concerned with absorption; the other a small cuboidal cell which contained fat only after feeding it and hence appeared to be concerned with excretion. No confirmation has been reported. A recent discussion of this question of resorption and excretion from the histologic point of view may be found in a paper by Winkenwerder.

Cholesterol can usually be demonstrated histologically in the mucosa of the normal human gall bladder and in the dog by the use of polarized light. If one assumes that its presence indicates absorption one must make the same assumption in regard to the common and hepatic ducts, for the mucous membrane lining these channels can also be shown to contain doubly refractive granules characteristic of cholesterol (2, 8, 19). Arndt in fact demonstrated them in dogs on a pure carbohydrate diet and when the surrounding liver cells showed no particle of this substance with the Nicol prisms. All in all histological study has failed to show how cholesterol behaves in the biliary tract, except that, if anything, it is excreted, not absorbed. Chauffard found no visible cholesterol in the bile duct epithelium.
of two dogs following ligation of the common duct, a condition favoring resorption whereas he saw it abundantly in two animals in which the ligature had cut through and bile was leaking out, a condition favoring excretion.

Further support for the hypothesis of excretion has been presented in preliminary studies from this laboratory (14-16) and will be described in detail in another paper (17). The experiments with cystic duct occlusion have already been mentioned. Another series of experiments in which comparisons were made between two specimens of bile collected from the same dog showed that the one subjected to gall bladder influence contained much more cholesterol than the other coming directly from the liver, even when the element of concentration of the bile was taken into account. In still other observations cholesterol was always found in the colorless secretion from the common ducts of dogs, and in the colorless hydrops fluid of the human gall bladder. Fowweather and Collinson also found cholesterol in the gall bladder contents of nine cases of hydrops as well as in the wall of the viscus, often in considerable amounts. Since these fluids are obviously the product of the biliary duct epithelium, the presence of the substance was an indication of its secretion. The present observations add further evidence in support of this view.

In this paper we shall present comparative analyses of gall bladder and hepatic bile from the same case, analyses from which, we believe, one may draw relatively accurate inferences.

Methods

Since the gall bladder has the power of concentrating bile it is necessary to measure this effect in some way. This was done by determinations of the bilirubin content, since this substance is excreted by the liver, and is indifferent to the activities of the bile duct epithelium at least under normal conditions or during short periods (38, 39). That is to say, if a particular specimen of gall bladder bile contained 3.0 mg. per cc. and the hepatic bile from the same case but 1.0 mg. per cc. it is obvious that the gall bladder had concentrated the bile three times and that if its cholesterol content was less than three times that of hepatic bile some had been absorbed; if more than three times some had been excreted.

In the analyses of human bile (Table I) the specimen from the gall bladder was obtained at operation; the hepatic bile was collected in the first three cases subsequently through the catheter placed in the common duct. Putrefaction was minimized by removing the accumulated secretion frequently and storing it in the ice box. In three cases sufficient bile was obtained for analysis by puncture of the common duct at operation, and in one case at autopsy.

In the analyses of dog bile (Table II) the gall bladder was removed and its contents were emptied immediately. The drainage of hepatic bile through intubation of the common duct was effected through the closed, 24 hour, aseptic method first described by Rous and McMaster (38). All bile specimens were centrifuged or filtered before analysis.
The chemical method for determination of bilirubin was that of Hooper and Whipple as modified by Rous and McMaster (39). Ordinarily 1 cc. of bile was added to 50 cc. of 95 per cent alcohol (containing 8 cc. concentrated HNO₃ and 40 cc. HCl per liter) and when the color turned green, was read in a colorimeter against an inorganic standard (10 cc. of 10 per cent CuSO₄ + 0.075 cc. of 1 per cent K₂Cr₂O₇) which in its color strength was equivalent to a solution containing 0.020 mg. of bilirubin per cc.

Cholesterol was determined by a modification of the Autenrieth-Funk method. Saponification was first effected by heating 5 cc. of bile with 20 cc. of 3 per cent KOH (in 95 per cent alcohol). Extraction was carried out in a separatory funnel with petroleum ether which was then evaporated. The residue was extracted with chloroform into 10 cc. volumetric flasks, 2 cc. acetic anhydride and 0.1 cc. concentrated H₂SO₄ added, made up to the mark, and the green color matched against a known cholesterol standard carried through the same color reaction. The details of this method are described at some length in a forthcoming paper (18).

FINDINGS

The results of our analyses are tabulated in the accompanying tables. Table I includes the human cases. These were patients operated on for cholecystitis with or without stones. It will be seen that the gall bladder bile contained much more cholesterol than the hepatic bile, the percentage differences being tabulated in the fourth column. That this increase could not be explained by concentration alone is shown by the bilirubin figures in the next columns, for the increase in cholesterol was much greater than that of bilirubin in all but one specimen. In three specimens, in fact, the hepatic bile was more concentrated than its corresponding gall bladder specimen. This is not surprising for the latter had come from a diseased organ which had lost most of its concentrating power by disease, and may even have diluted the bile by an abnormal secretion of mucus.

In four cases the comparisons were made between specimens obtained simultaneously, (three at the time of operation and one at autopsy) for it was possible in them to obtain enough hepatic bile for analysis by puncture of the common or hepatic duct. The findings are of special importance for this reason and in each case showed, as in the others, a greater cholesterol content in the gall bladder bile than can be accounted for by inspissation alone. It may be objected that hepatic bile obtained by puncture of the common duct may have been mixed with gall bladder bile passing into it through the cystic duct.
TABLE I

Showing the Actually and Relatively Greater Cholesterol Content of Human Gall Bladder over Hepatic Bile in the Same Patient

<table>
<thead>
<tr>
<th>Case</th>
<th>Cholesterol Concentration of bile (Bilirubin)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hepatic bile</td>
<td>G.B. bile</td>
</tr>
<tr>
<td></td>
<td>mg. per cc.</td>
<td>mg. per cc.</td>
</tr>
<tr>
<td>J. S.</td>
<td>3.7</td>
<td>0.50</td>
</tr>
<tr>
<td>M. J.</td>
<td>0.30</td>
<td>0.11</td>
</tr>
<tr>
<td>C. S. H.</td>
<td>0.28</td>
<td>0.21</td>
</tr>
<tr>
<td>R. L.</td>
<td>0.45</td>
<td>0.28</td>
</tr>
<tr>
<td>L. L. E.</td>
<td>0.74</td>
<td>0.63</td>
</tr>
<tr>
<td>M. R.</td>
<td>0.91</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Gall bladder bile from gall bladder removed at operation. Hepatic bile from puncture of common duct at operation. Both biles contained cholesterol sediment; both filtered before analysis

Gall bladder bile from gall bladder removed at operation. Hepatic bile aspiratec from common duct on July 9, 2017.
### TABLE I—Concluded

<table>
<thead>
<tr>
<th>Case</th>
<th>Cholesterol</th>
<th>Concentration of bile (Bilirubin)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic</td>
<td>ms. per cc.</td>
<td>G.B. bile</td>
<td>ms. per cc.</td>
</tr>
<tr>
<td>bile</td>
<td>per cent</td>
<td>bile</td>
<td>per cent</td>
</tr>
<tr>
<td>J. B.</td>
<td>0.94</td>
<td>1.35</td>
<td>+97</td>
</tr>
</tbody>
</table>

### TABLE II

**Showing the Actually and Relatively Greater Cholesterol Content of Gall Bladder over Hepatic Bile in the Same Dog (Normal Secretions)**

<table>
<thead>
<tr>
<th>Dog</th>
<th>Cholesterol</th>
<th>Concentration of bile (Bilirubin)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic</td>
<td>ms. per cc.</td>
<td>G.B. bile</td>
<td>ms. per cc.</td>
</tr>
<tr>
<td>bile</td>
<td>per cent</td>
<td>bile</td>
<td>per cent</td>
</tr>
<tr>
<td>21</td>
<td>0.19</td>
<td>0.064</td>
<td>+230</td>
</tr>
<tr>
<td>15</td>
<td>0.075</td>
<td>0.35</td>
<td>+370</td>
</tr>
<tr>
<td>10</td>
<td>0.040</td>
<td>0.25</td>
<td>+511</td>
</tr>
<tr>
<td></td>
<td>0.17</td>
<td>1.00</td>
<td>+490</td>
</tr>
</tbody>
</table>
by pressure during the operation or by contraction of the viscus. If this really did occur it would have minimized the differences found and correction for it, if possible, would make the findings more striking as evidence that the gall bladder excreted cholesterol.

The findings in similar analyses in dogs are summarized in Table II. The specimens were all sterile. Here the same differences noted in man were also found. The gall bladder bile in each case contained much more cholesterol than the hepatic bile, even if we allow for inspissation of the bile as measured by the bilirubin content. In two of the dogs, in fact, the gall bladder bile was less concentrated than the liver bile. This was true because these dogs had not been fasted and the operation was performed during the height of digestion, a time during which gall bladder bile is always dilute. Yet, even in the absence of concentration the cholesterol content was quite high, an observation which we have made in numerous analyses of isolated specimens of normal dog gall bladder biles. The values for the hepatic bile were obtained by averaging the results of many determinations. In this way changes due to the operation, diet, etc. were somewhat leveled out. It must be admitted, however, that these biles, collected after an operation, are not normal like the gall bladder bile removed from the intact gall bladder. The differences found, however, were so striking that one cannot escape the inference that cholesterol is added to the bile by the gall bladder wall.

The values recorded in these experiments for hepatic bile in the dog are somewhat lower than those reported by Doyon and Dufourt, and considerably lower than those reported by Jankau. Both of these observers used older methods of analyses however. Enderlen, Thannhauser, and Jenke using the digitonin method find lower values for dog bile which agree fairly well with our results (0.08 to 0.2 mg. per cc.). Stern in 1928 found a similar range of values. McMaster’s values however were, in general, somewhat higher than ours.

DISCUSSION

The analyses reported herein are the first ones (except those on the dog of Doyon and Dufourt) in which gall bladder and hepatic bile from the same source have been compared. Errors due to cellular or other debris were ruled out by filtering all specimens, and the concen-
trafing influence of the gall bladder was accounted for by bilirubin determinations in each case. That in spite of these corrections the gall bladder bile still showed the greater content of cholesterol points to the wall of the viscus as its probable source. A possible objection to such an inference may be that the compared specimens, though from the same source, were not collected simultaneously, that one should really have a sample of the hepatic bile which had gone into and had been acted upon by the gall bladder, rather than that which was secreted afterwards. This would introduce a considerable error only if the liver bile varies greatly from day to day, a condition which does not occur normally. This objection applies least to the four human cases in which the two specimens were obtained simultaneously. In the case of the dog averages of many hepatic bile samples probably corrected for some of such errors. But the objection is a valid one and the findings have value as suggestive evidence only because they are so marked and consistent. Taken moreover with the results of the other experiments already pointed out, the inference seems justified that cholesterol is excreted by the gall bladder mucosa.

Since biliary epithelium is derived from the intestine this phenomenon is not surprising. Evidence has been accumulating that the intestinal tract and not the bile is probably the main site of origin (in addition to the food) of sterols found in the stool. Thus Sperry has shown that in dogs with bile draining to the outside and fed a cholesterol-free diet, the fecal output of this substance is maintained and even increased. Loops of bowel isolated from the rest of the intestinal tract have been shown to excrete preceptible amounts of fat (1) and while cholesterol was not tested for, it seems likely that it was present.

That infection increases the excretion of cholesterol has been suggested in previous experiments. Inflammatory exudates have long been known to contain large amounts of it (49). Herter and also Thomas have shown that inflammation of the gall bladder and bile ducts leads to a marked increase in cholesterol content of the bile they drain. And Illingworth was able to produce cholesterosis of the gall bladder by infecting that organ in rabbits while they were on a high cholesterol diet. It has been generally assumed that this increase coincidental with infection is due to the presence of degenerating
cells which contain cholesterol (49). There is some evidence to show that this is not entirely true (50). Although it would seem that inflammation can increase the output of cholesterol in the cell-free contents of the inflamed gall bladder or biliary tract its final proof must remain for further study.

The temptation to apply the present findings to the long discussed problem of the formation of gall stones cannot be easily resisted. If we correlate them with certain observations of others an attractive hypothesis is suggested. Thus Rosenthal and Licht have recently shown that bile salts are normally absorbed by the gall bladder wall and that inflammation accelerates this absorption, a mechanism directly opposite to the one we believe is true for cholesterol. The rôle of bile salts in keeping cholesterol in solution is plain from many observations. Oliver for example has studied 75 cases of human gall bladder bile and found that spontaneous cholesterol precipitation on standing occurred only in those with a low bile acid content and never in those with a normal or high content. With this in mind conditions leading to the precipitation of cholesterol could theoretically develop even on the basis of stasis alone. Thus bile by remaining in the gall bladder loses bile salts and gains cholesterol, conditions which if prolonged might lead to a relative supersaturation of the latter and then perhaps to its eventual precipitation.

The oft disputed question as to whether stasis alone may actually be responsible for the formation of gall stones cannot be answered on the basis of our present observations, suggestive though they are, inasmuch as certain clinical facts seem to argue against it. Thus it has been pointed out by Sherwood Moore that gall stones are rarely found in the type of patient who, by cholecystography, shows stasis in the gall bladder. This individual, of the thin asthenic habitus, often fails to empty his gall bladder for 36 hours following a cholecystogram despite a fat meal. Yet he is not the person who is apt to suffer from gall stones. It is the robust, hypersthenic one, whose gall bladder empties quickly, who most frequently has cholelithiasis. Again, we rarely see gall stones form in the thick tarry bile removed from the dilated gall bladder of patients with malignant obstruction of the lower end of the common duct, though here stasis is prolonged and complete.
It may be necessary therefore to invoke the aid of inflammation after all and the evidence mentioned seems to favor this possibility for the excretion of cholesterol as well as the absorption of bile salts by the gall bladder seems to be accelerated in the presence of infection. Various other factors are also concerned in the solution and precipitation of cholesterol, a discussion of which can be found in the paper of Lichtwitz. More recently the reaction of the bile has been shown to be of considerable importance (12) and it may be that only after the importance of all these factors are evaluated will the true pathogenesis of gall stones be revealed.

The development of a "strawberry" gall bladder is also easy to explain on the excretion theory and will be discussed at some length elsewhere (17). Cholesterol accumulates in the walls of these gall bladders presumably because the bile can no longer take up any more of it. Indeed the few analyses of gall bladder bile from cases of "strawberry" gall bladder (our own and those of Illingworth) show a high cholesterol content. Unfortunately no analyses of bile salts on these cases have been made. Corroborative of this theory too are the extensive analyses of Fowweather and Collinson who found a "mean" cholesterol value in the bile of diseased gall bladders of 3.5 mg. per cc. as compared with the normal of 2.3. They also found that the diseased gall bladder itself contained more cholesterol i.e. 1.20 mg. per cent against the normal of 0.91.

These studies as well as others elsewhere enable us to picture the gall bladder not as a simple viscus absorbing water and excreting mucus but as also excreting cholesterol, absorbing bile salts (37), calcium (13, 33), and as making the bile more acid (12). To explain fully its various pathological features we must take into account all these functions, for perversion of one or more may lead to a variety of results. From a simple concentrating reservoir it would seem that the gall bladder is coming to attain the manifold activities of a real abdominal organ.

SUMMARY

Cholesterol determinations of gall bladder and hepatic bile obtained from the same source reveal a greater concentration in the former even after the inspissating effect of the gall bladder is allowed for. This
evidence together with that from other experiments indicates that the
gall bladder has the power to excrete cholesterol into its lumen. There
is evidence also that infection may accelerate this excretion. An
hypothesis is presented to explain the precipitation of cholesterol in
the bile, and the bearing of these findings on the pathogenesis of
cholesterol stones is briefly discussed.

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