

## STUDIES ON GALL BLADDER FUNCTION

### VIII. THE FATE OF BILE PIGMENT AND CHOLESTEROL IN HEPATIC BILE SUBJECTED TO GALL BLADDER ACTIVITY

By CECILIA RIEGEL, PH.D., C. G. JOHNSTON,\* M.D., AND I. S. RAVDIN, M.D.

(From the Laboratory of Research Surgery, University of Pennsylvania, Philadelphia)

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Eleven years have passed since Rous and McMaster (15) published their paper on the concentrating activity of the gall bladder. This contribution and subsequent ones by them and their associates renewed the interest of investigators in this organ. Two schools have developed, each with a different fundamental concept of gall bladder function. The one, following what would appear to be the more logical concept, maintains that concentrated bile is emptied at intervals into the duodenum. The other, largely from teleological reasoning, believes that the cystic duct is a one-way tube, permitting hepatic bile to flow into the gall bladder, but preventing it from flowing out. Those who accept the latter concept must necessarily adhere to the theory that every constituent of the bile is absorbed through the gall bladder wall. If the latter group is correct there must be an optimum concentration for different constituents of the bile at which level they are absorbed at a definite rate.

The experiments of Rous and McMaster indicated that bile pigment was not absorbed through the gall bladder wall. If this is true, and if the gall bladder has no other way of evacuating itself except by absorption, it would not take long for the lumen to be filled with an inspissated mass of pigment. The experiments on the pigment changes of hepatic bile subjected to gall bladder activity were open to certain errors which Rous and McMaster duly considered. The experiments were not absolutely quantitative in that the amount of hepatic bile entering the gall bladder could not be absolutely measured.

\* Harriet M. Frazier Fellow in Research Surgery.

It was thought that a modification of their method might offer additional evidence on the fate of bilirubin in the gall bladder.

There has been considerable controversy in the literature on gall bladder function as to whether cholesterol is absorbed from, secreted into, or merely concentrated in gall bladder bile. The polemic is an old one, having been carried on for years by Aschoff (2) and Naunyn (12). The literature on this subject has been fully reviewed (6, 9, 10). Data on this controversial point are important because of the relationship which cholesterol bears to calculus formation. Recent papers published by Elman and Taussig (5, 6), and Elman and Graham (4) support the Naunyn hypothesis in that these workers present evidence which would appear to show that the normal gall bladder secretes cholesterol into the gall bladder bile.

Data which we have accumulated do not agree with this point of view. The differences should be easily reconciled if a method can be devised which will permit quantitative studies in a normally functioning gall bladder. We have recently described such a method (13). It permits quantitative studies in a gall bladder which is free of any ductal connection with the liver, but which retains its normal blood supply and major lymphatic vessels. The animals are normal in that a large proportion of the bile from the liver still passes into the intestine. The appetite is unaltered and nutrition does not suffer.

#### *Method*

The method of intubation of the gall bladder has been sufficiently described (13). It consists in isolating the gall bladder from any ductal connection with the liver and inserting a soft Nélaton catheter through a slit in the hepatic duct just above the entrance of the right and left lobe ducts. The catheter is passed through the cystic duct to a position just inside the lumen of the gall bladder. When it is *in situ* a ligature is passed around the common hepatic duct and tied so as to hold the catheter in position. The catheter is brought out through a stab wound to either side of the incision.

The animals remain in excellent condition after operation.<sup>1</sup> They are dressed with the same care used during the operation in order to maintain asepsis.

This method has been used in this laboratory for over 2 years during which period we have been convinced that the preliminary operative

<sup>1</sup> The operations were done under ether anesthesia.

procedure does not alter normal function. Repeated injection of the lymphatics after this procedure has shown that the major lymphatic drainage from the gall bladder is not disturbed. The cystic artery and vein are never damaged. The gall bladder is not handled during the operation. Every visible accessory duct is ligated if this can be done without damage to the gall bladder lymphatics or blood vessels; otherwise the animals are discarded. The studies on bile have all been made on the unanesthetized animal.

Every preparation is tested for the presence of unligated accessory ducts and for the ability of the gall bladder to absorb water by placing a known amount of normal sodium chloride in the gall bladder after having cleansed it and permitting the solution to remain for from 1 to 3 hours. If accessory ducts have been overlooked, the clear solution becomes bile-tinged. Furthermore, every animal has been carefully autopsied after the injection of methylene blue into the gall bladder. This serves as an added check on the presence of accessory ducts. These are so frequent in the dog that any experiment which fails to consider their presence is of doubtful value. We have recently completed anion-cation studies of hepatic bile subjected to gall bladder activity and have found that the organ as prepared by our method will alter the hepatic bile in composition so that the bile removed is nearly identical with the gall bladder bile removed from the organ at the time of intubation.

The hepatic bile was obtained from cholecystectomized dogs after double catheterization of the common duct (14). Since the bile was never collected for longer than 24 hours before it was again turned into the duodenum, the animals remained in excellent condition.

Cultures and smears were made constantly of the bile of the animals of each group and, as soon as infection occurred, they were discarded. Rigid asepsis is necessary in any experiment of this type.

The bile pigment was estimated by the Hooper and Whipple method (7) with the modification of the inorganic standard as suggested by Rous and McMaster (15). A series of standards was found advantageous for close comparison with the unknowns. Furthermore, since nearly all of the determinations were made during the summer months, it was necessary to keep the tubes at a constant temperature for a definite period after the introduction of the acid alcohol. Controls run at the beginning of the experiment showed us that the maximum depth of the bluish green color developed after 4 hours at 38°C. This time and temperature were therefore adopted for all determinations. Merely waiting until the color turns green, as recently suggested (6), results in inaccurate estimations of the pigment present. The color turns bluish green long before the maximum intensity is developed. Occasionally the color becomes purplish or grayish so that comparisons are not possible. Using known amounts of pigment, the error of the Hooper and Whipple method has been about 5 per cent in our hands.

All bile pigment readings were made with a monochromatic neon lamp provided with a Corning filter. The neon tube was 32 inches long of  $\frac{1}{4}$  inch Pyrex tubing doubled on itself eight times. The filters were heat-resisting, red, 3.95 mm. thick, and heat-absorbing, 2.95 mm. thick, of medium shade.

The cholesterol was determined by the method of Autenrieth and Funk, as described by McMaster (11) with readings against a known cholesterol standard. The error in the determination of known amounts in our hands has been from 10 to 15 per cent. The method is far from ideal.

#### RESULTS

*Bile Pigment.*—Known amounts of hepatic bile of a determined bile pigment concentration were introduced into the gall bladder and permitted to remain there for from 2 to 24 hours. In some of the experiments further additions of hepatic bile were made from time to time so that the total amount of hepatic bile received by the gall bladder was frequently several times that which the organ originally held. When the bile was withdrawn, its amount was carefully determined and the gall bladder was then washed out twice with normal saline. The concentration of the pigment in the specimen and in the washings was then determined and the two added in order to find the total amount recovered.

Table I shows the changes in fluid content after hepatic bile was placed in the gall bladder. These vary considerably because of difference in the time when the last hepatic bile was introduced. From 64 to 93.4 per cent of the introduced fluid was removed in a 24 hour period.

It is evident that in those instances in which the amount of pigment withdrawn exceeded the amount introduced the increase was small and was within the error of the method. In sixteen of the eighteen experiments there was a small apparent loss of bile pigment. In only three instances (Dogs 521, 44, and 45) did the amount lost exceed the error of the method and then only very slightly. In these experiments the fluid change was from 26.5 to 2.5 cc., 32.5 to 7 cc., and from 25 to 4.5 cc. respectively. The pigment loss was therefore relatively small in comparison to the fluid loss of 90.6, 81, and 86 per cent.

Table II gives the results of eleven experiments in which hepatic bile was placed in the bile-free gall bladder and cholesterol determinations made. The cholesterol concentrations of the introduced material

varied from 11 mg. per 100 cc. to 46 mg. per 100 cc. of bile. These concentrations are all below those of cholesterol in dogs' blood. The concentrations in the bile removed from the gall bladder after the

TABLE I  
*Bile Pigment Changes in Hepatic Bile Subjected to Gall Bladder Activity*

Dog No.	Time	Concen- tration in hrs. gm./100 cc.	Concen- tration out gm./100 cc.	Volume in cc.	Volume out cc.	Amount in mg.	Amount out mg.	Amount change mg.	Change per cent
1134	2	1.61	5.29	18.5	5.0	28.98	26.45	-2.53	-8.7
1134	18	1.70	9.16	18.0	2.5	30.60	27.60	-3.00	-9.8
1134	2	0.39	0.53	15.0	11.0	5.85	5.83	-0.02	-0.3
1134	2	0.55	0.56	13.0	11.5	7.15	7.13	-0.02	-0.2
1134	16	0.32	2.59	13.0	1.5	4.16	3.89	-0.27	-6.4
1119	2	0.35	0.76	20.0	6.5	7.00	6.29	-0.71	-10.1
15	22	1.80	—	15.0	1.0	27.00	24.43	-2.57	-9.5
521	24	1.02	—	26.5	2.5	27.03	23.80	-3.23	-11.9
44	24	0.56	1.90	32.5	7.0	18.20	15.90	-2.30	-12.6
40	24	0.39	1.86	18.5	3.5	7.22	7.53	+0.31	+4.2
45	24	0.83	3.00	25.0	4.5	20.75	18.06	-2.69	-12.9
6	24	1.20	3.70	35.0	9.7	42.00	39.99	-2.01	-4.7
6	2	2.20	2.10	20.0	17.5	44.00	44.49	+0.49	+1.1
60	24	0.82	2.70	18.0	4.5	14.76	14.07	-0.69	-4.6
184	7	0.86	1.20	35.0	24.0	30.10	29.80	-0.30	-0.9
184	17	1.02	1.50	40.0	26.0	40.80	39.60	-1.20	-2.9
186	12	0.38	0.66	20.0	9.0	7.60	7.49	-0.11	-1.4
186	2	0.32	0.49	20.0	12.5	6.40	6.73	-0.33	-4.9
Mean.....								-5.36	
Median.....								-4.8	

hepatic bile had been subjected to gall bladder activity for a variable period ranged from 25 to 141 mg. per 100 cc., the latter approaching the blood level. In only one experiment in which the gall bladder was

washed was there an increase in the total amount of cholesterol removed over the amount introduced, and this was well within the error of the method. The mean difference between the amount of cholesterol introduced and the amount removed at the end of the experiment was 10.5 per cent. In every experiment except one the percentage removal of fluid was considerably greater than the percentage loss of cholesterol. In this experiment, Dog 65, the bile placed in the gall

TABLE II  
*Cholesterol Changes in Hepatic Bile in the Gall Bladder*

Dog No.	Time	Concen- tration in hrs. gm./100 cc.	Concen- tration out gm./100 cc.	Volume in cc.	Volume out cc.	Amount in mg.	Amount out mg.	Amount change mg.	Change per cent
702	30	0.046	0.141	49.0	15.5	22.5	23.5	+1.0	+ 4.4
642	21½	0.026	0.121	50.5	6.8	13.1	11.3	-1.8	-13.7
642	24	0.026	0.062	33.0	8.0	8.6	6.8	-1.8	-20.9
737	24	0.026	0.087	35.0	8.5	9.1	8.4	-0.7	-7.6
190	6	0.019	0.031	40.0	16.0	7.6	5.0	-2.6	-12.7*
190	12	0.016	0.050	60.0	20.0	9.6	10.0	+0.4	
218	5	0.027	0.096	20.0	7.5	5.4	7.2	+1.8	
218	12½	0.027	0.140	20.0	3.0	5.4	4.2	-1.2	-18.1*
218	7	0.026	0.029	20.0	6.0	5.2	1.7	-3.5	
65	24	0.123	0.124	10.0	9.5	12.3	11.8	-0.5	-4.0†
1123	24	0.011	0.025	48.75	19.0	5.4	4.8	-0.6	-11.1

\* No washings.

† Gall bladder bile.

bladder was the gall bladder bile from another dog. The loss of fluid in this experiment was quite small, as would be expected.

In order to show the error which may arise if accessory ducts are not carefully excluded, Table III is presented. In every instance tabulated, one or more accessory ducts existed which were not seen at operation. Some of these entered the gall bladder directly while others emptied into various portions of the cystic duct. Thus hepatic bile was being added to the gall bladder over and above the amount

which we were introducing artificially. The concentrations of cholesterol in the bile introduced varied from 12.0 to 26.0 mg. per 100 cc., while that of the bile removed from the gall bladder varied from 35.0 to 150 mg. per 100 cc. In every instance there was an increase in the total amount of cholesterol removed, although in every other respect (loss of fluid, concentration, etc.) the results were quite similar to those obtained from animals whose gall bladders had no accessory ducts. The increase in the total amount of cholesterol was above the amount which could be explained by the error of the method of determination.

That infection of the gall bladder may cause the cholesterol recovered to be greater in amount than that introduced is shown in the following

TABLE III  
*Cholesterol—Dogs with Accessory Ducts*

Dog No.	Time	Concen- tration <i>in</i> hrs. gm./100 cc.	Concen- tration <i>out</i> gm./100 cc.	Volume <i>in</i> cc.	Volume <i>out</i> cc.	Amount <i>in</i> mg.	Amount <i>out</i> mg.	Amount change mg.	Change per cent
610	21	0.012	0.082	40.0	7.3	4.8	6.9	2.3	+47.9
610	48	0.012	0.082	56.0	10.5	6.7	8.6	1.9	+28.3
639	30	0.020	0.150	56.0	13.5	11.2	23.7	12.5	+111.6
640	17	0.026	0.132	20.0	2.3	5.2	6.2	1.0	+19.2
640	24	0.026	0.055	40.0	26.0	10.4	14.3	3.9	+37.5

experiment.—Dog 190. After infection of the gall bladder wall we obtained a 96 per cent increase in the total cholesterol in a 24 hour period. The total amount of cholesterol introduced was 12 mg. while the total amount removed was 23.5 mg.

#### DISCUSSION

As already stated, studies in this laboratory have shown us that the type of preparation we are using provides a normal gall bladder. Hunt, Davis, and Boyden (8) have shown that ligation of the cystic duct or tying the cystic mesentery in such a way as to produce stasis in the gall bladder, even though the arterial circulation is not interrupted, results in cholecystitis. The changes which they found

ranged from the mildest inflammation to hydropic distention of the gall bladder.

The disappearance of bile pigment during an inflammatory reaction of the gall bladder wall cannot be taken as evidence that, under normal conditions, the same thing will occur. In Dog 1119, after infection, 33.8 per cent of the bile pigment was lost from the gall bladder in a 2.5 hour period and, in Dog 17, 36 per cent of pigment was lost in a 2 hour period. We do not know whether this loss is due to absorption of the pigment or to a change in its composition making quantitative determination impossible.

Several facts are obvious from an analysis of our bile pigment data. If the bile is merely removed from the gall bladder without rinsing the organ, the amount of pigment recovered may be considerably lower than one would expect from the percentage of fluid lost from the gall bladder. In fact the pigment concentration may appear to be but slightly changed from that of the bile introduced. The amount recovered in the washings makes plain the fact that pigment clings to the gall bladder wall. Since the gall bladders used in these studies were carefully cleansed before the introduction of the hepatic bile, the pigment attached to the wall at the conclusion of the experiment could only have come from the bile introduced. A considerable amount of this can be removed by repeated aspiration and reinjection of the bile, as was done in Dog 1134, but the results vary. Therefore we finally adopted the method of merely aspirating the contained bile with a Luer syringe and subsequently washing the gall bladder with a measured amount of saline.

Undoubtedly the low pigment concentrations which are often reported for gall bladder bile are the result of a failure to recover the total pigment present. It is impossible, even by the method which we used, to recover quite all the pigment introduced since washings subsequent to those used in the final determination give dilutions so low as to make analyses untrustworthy. The mean loss of pigment in the eighteen experiments was 5.36 per cent and the median was 4.8 per cent.

We do not believe that one can assume from the data presented that any pigment is absorbed. Furthermore, we have repeatedly cannulated the cystic lymph vessels as did Rous and McMaster (15) and

have failed to demonstrate the presence of any bile pigment in the lymph from the gall bladder wall.

A difference of opinion still exists as to whether cholesterol is absorbed by the gall bladder mucosa (Aschoff), or is secreted by it (Naunyn). Among the more recent work supporting the absorption theory may be mentioned that of Torinoumi (17), Illingworth (9), Boyd (3), and Andrews, Schoenheimer, and Hrdina (1). Boyd (3) did not actually measure the cholesterol content of gall bladder bile, but drew his conclusions from histologic studies and from changes in the cholesterol content of the blood after cholecystectomy. Illingworth (9) concluded from experiments on two cats that when cholesterol is present in excess it can be absorbed. He gave no data on the amount of fluid in the gall bladder at the time of autopsy. The amount which may have precipitated out of the emulsion was not considered. If the contents increased considerably during the period of study it is likely that the gall bladder was not normal.

Torinoumi (17) found that cholesterol was absorbed from the normal gall bladder and secreted into the infected gall bladder. Andrews, Schoenheimer, and Hrdina (1) agree with these findings, although they have published no data to prove absorption in the normal animal. Elman and Taussig (5) found that, after ligation of the cystic duct, both the concentration and the total amount of cholesterol in the gall bladder were increased as a rule.

Elman and Taussig (6) and Elman and Graham (4) have more recently reported cholesterol analyses of hepatic and gall bladder bile from the same animal. The method which they used for estimating the amount of bile flowing into the gall bladder was the partitioning ligature method used by Rous and McMaster (15). This method is not an exact quantitative method since the amount of bile secreted per gram of liver tissue may not be the same under the conditions of the experiment. Furthermore, no mention is made of the exclusion of accessory biliary ducts. Failure to eliminate these may give results similar to those which Elman and his associates have presented for apparently normal animals.

While Rous and McMaster (15) have shown that bile pigment under normal conditions is excreted in nearly uniform concentrations from different portions of the liver, there are no data upon which one can base the assumption that every other constituent of the bile is similarly excreted. Furthermore, the experiments of Elman and Taussig (6) which use bile pigment as a measure of gall bladder activity fail to show concentration of bile pigment in two of the four experiments, a condition we have never encountered in the normally functioning gall bladder of the dog.

From histologic studies made in this laboratory, and from the observations of Hunt, Davis, and Boyden (8) it would appear useless

to consider as normal the cholesterol figures obtained from animals whose cystic duct has been ligated. It would seem equally useless to discuss any data in which the numerous accessory ducts entering the cystic duct or gall bladder had not been taken into account in estimating the total bile received by the gall bladder.

The increases in cholesterol content which Elman and Taussig (6) and Elman and Graham (4) have found in human gall bladder bile were from obviously diseased gall bladders. That cholesterol may increase when infection or inflammation is present cannot be doubted. The data presented from Dog 190 after infection had occurred agree with their findings. One cannot assume, however, that the gall bladder membrane acts in exactly the same manner when normal and when diseased.

#### SUMMARY

The data obtained from the experiments on dogs reported in this paper lead us to conclude that bile pigment is not absorbed from the gall bladder bile. The mean loss of pigment is so small when compared with the amount of water lost that it is negligible.

Our cholesterol data do not support the concept that this substance is secreted into the gall bladder bile of the dog under normal conditions. In the majority of experiments there was a loss of cholesterol. Indeed we have failed to find any evidence of definite secretion or absorption save in the case of the infected gall bladder. We are led to conclude, as did Rous and McMaster (15) with regard to bile pigment, that normally there is no absorption of cholesterol.

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