STUDIES ON GALLBLADDER FUNCTION. IX. THE ANION-CATION CONTENT OF BILE FROM THE NORMAL AND INFECTED GALLBLADDER

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In an earlier paper (1) we discussed the anion-cation concentration of normal hepatic bile when subjected to the activity of the dog's normal gallbladder. Under these conditions the chloride and bicarbonate concentrations and the pH of hepatic bile decrease, while the base, bile salt and calcium concentrations increase. In every instance fluid is absorbed. In a very limited number of observations we have found the depression of the freezing point of hepatic and gallbladder bile approximately the same, although the total anion-cation concentration was increased considerably in the latter.

As a prelude to the anion-cation studies we had studied the effect of gallbladder activity on certain of the individual constituents of hepatic bile when placed in the normal bile-free gallbladder of the unanaesthetized dog (2) (3) (4). It was observed that when the gallbladder became infected the activity of the membrane on the constituents studied was altered considerably. In such instances, water was only slowly absorbed; or, in the more severely damaged organ, fluid actually poured into its lumen, the latter action being a complete reversal of the normal mechanism. Under these circumstances chloride and bicarbonate entered the gallbladder lumen with the inflowing fluid, the chloride concentration of the secreted fluid being about plasma level while the total CO_2 concentration was often several times the normal plasma level. Calcium, introduced as calcium lactate, was precipitated, partly in the gallbladder lumen, and partly in the gallbladder wall. Cholesterol in either a colloidal suspension, or in hepatic bile, increased in concentration, but not in total amount when placed in the normal gallbladder. The same was true of bile pigment. When, however, the gallbladder was infected the total amount of cholesterol increased, while the total amount of bile pigment decreased (5).

These isolated observations made at a time when we were chiefly concerned with normal function convinced us that a study of the anion-cation changes in hepatic bile subjected to the activity of an abnormal

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gallbladder was necessary in order to interpret the changes which occur in disease of that organ.

METHOD

The initial studies were made on the dog using a preparation similar to that which we have previously described (2), except that after convincing ourselves that the gallbladder was functioning normally, we either intentionally or unintentionally permitted the gallbladder to become infected. The usual infecting organism was a non-hemolytic streptococcus. In the studies on the dog, known amounts of normal dog's hepatic bile were placed in the infected bile-free gallbladder. After varying intervals the bile was recovered and the various constituents of the bile were determined by the methods reported in previous papers (1) (2) (3) (4) (5). The dogs behaved normally, and beyond the infection of the gallbladder, no evidence of disease existed.

In the human cases the bile was removed from the gallbladder at the operating table. It was transferred to a test tube aseptically and the analyses were made as soon as it was possible to transfer the bile to the laboratory. Some of the patients were operated on under local anaesthesia, some under spinal, while others received nitrous oxide and oxygen plus a small amount of ether.

Some studies have been made on liver bile obtained after drainage of the common duct for obstruction. The bile specimens so obtained were collected in receptacles for 24 hours when samples were removed for analyses. Successive samples were analyzed until evidence was obtained that the liver bile was approaching what we thought was a normal liver bile, or until the surgeon removed the common duct drainage tube, making further specimens unobtainable.

TABLE I

Analysis of the gallbladder bile from the normal and infected gallbladder of the dog

Dog number	Date	Bile in	Bile out	Bile removed					
				Base	Calcium	Chloride	$BHCO_3$	Bile salt	pH
		cc.	*cc.*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	
490	* November 23–24, 1931	96.5	22.0	240	31.1	2.1	6.7	252.0	
	† January 26–27, 1932	14.0	29.0	184	5.2	111.2	47.2	2.1	7.5
	† January 31–February 2, 1932	5.0	29.0	184	6.6	129.3	37.4	0.0	7.8
410	* November 17–18, 1931	57.0	8.8	241	26.4	8.2		282.0	
	† November 20, 1931	10.0	28.0		11.2	78.9	52.5	12.9	
203	* September 21, 1931	23.0	8.0		17.1	37.2	7.3	168.4	6.6
	† September 22, 1931	30.0	34.0	169	7.0	85.4	40.2	22.4	7.5

* Gallbladder normal.

† Gallbladder infected.

RESULTS

In Table I are given data from several animals before and after gallbladder infection had taken place. In the normal gallbladder the concentration of chloride and total CO_2 decreased very markedly, as did the total fluid volume. Since hepatic bile was added at intervals to the gallbladder contents the relation of time to total fluid introduced and removed was quite variable. The volumes reported merely indicate direction rather than rate of change. With a single injection of bile we have found that the gallbladder will concentrate the fluid volume in a 24 hour period to as little as one-sixteenth the original amount introduced. Furthermore, in the normally functioning gallbladder the pH of the bile uniformly decreased, while the base, bile salt and calcium concentrations increased.

When the gallbladder became infected there occurred a nearly complete reversal of its activity. The chloride and total CO_2 concentrations were uniformly higher. In the infected gallbladder the pH of the bile increased. In contrast, the base, bile salt and calcium concentrations decreased. The tremendous loss of bile salt under such conditions is indeed striking. Fluid volume, instead of decreasing, increased, indicating not only a failure of absorption but an actual pouring of fluid into the gallbladder lumen. Since no bile could enter the gallbladder except that which was placed in it the additional fluid must have come from the vessels of the gallbladder wall.

The data from the human gallbladder bile fall into several groups. The specimens were analyzed, without our knowledge in many instances of the clinical findings. After analysis the surgeon was asked for the roentgenologic and operative findings, and it was soon observed that the data were roughly divisible into several groups.

No data were found in the literature based on analyses of normal human gallbladder bile. It was important to obtain such data if a comparison was to be made with the dog's gallbladder bile. We secured one specimen of gallbladder bile by aspiration from a patient whose abdomen was opened under local anaesthesia and a second specimen was

TABLE II

Comparison of normal human and dog's gallbladder bile

Dog number	Base	Calcium	Chloride	Bile salt
	m. eq. per liter	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*
410		22.4	10.3	188.8
472		25.5	3.9	304.0
446		27.6	4.7	289.0
Patient				
D.	298	28.5	16.9	208.0
E.		25.9	18.4	152.0

supplied to us by Dr. E. L. Eliason from a patient operated on under nitrous-oxide-oxygen-ether anaesthesia (Table II). Neither of these cases had any evidence of biliary tract disease. These gallbladders at operation were thin and blue, and free of any adhesions. It will be readily seen that there is a fairly close parallelism between the normal gallbladder bile of man and dog, as regards the constituents which we are reporting.

TABLE III

Analysis of gallbladder bile of human cases. Chronic cholecystitis—no stones. Gallbladder visualized after administration of sodium tetraiodophenolphthalein

Patient	Base	Calcium	Chloride	Bile salt
	m. eq. per liter	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*
R.		12.1	57.6	99.8
S.	290	24.0	17.1	99.3
D.	263	15.2	41.8	79.0
B.	220		69.3	62.0

TABLE IV

Analysis of gallbladder bile of human cases. Chronic cholecystitis—with stones. Gallbladder visualized after administration of sodium tetraiodophenolphthalein

Patient	Base	Calcium	Chloride	Bile salt
	m. eq. per liter	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*
E.		18.3	39.5	81.0
G.		20.8	52.0	78.3
A.	189	12.1	68.1	52.4

TABLE V

Analysis of gallbladder bile of human cases. Chronic cholecystitis—with stones. Gallbladder not visualized after administration of sodium tetraiodophenolphthalein

Patient	Base	Calcium	Chloride	Bile salt
	m. eq. per liter	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*
P.		10.7	115.6	52.0
Be.	199		63.6	42.0
C.		9.5	60.9	32.3
M.				26.0
Br.	151	3.0	140.7	3.4
L.		4.5	93.5	1.8
H.	151	10.0	112.7	0.0
T.	121	5.3	80.1	0.0
S.	146	7.2	103.4	0.0
W.		1.6	152.2	0.0
Sa			81.1	0.0
Mc.		2.8	82.5	0.0

In Tables III, IV and V are given data from specimens of pathological human gallbladder bile. In the cases in Groups III and IV the gallbladder was visualized after administration of sodium tetraiodophenolphthalein. Visualization was not obtained in Group V. It is difficult to state quantitatively the degree of visualization since the technics employed in the administration of the dye may have varied. By visualization we mean that the roentgenologist was able to see the gallbladder outline, but this may or may not have been considered entirely normal.

As a whole these data show a decrease in the calcium concentrations, the lowest concentrations occurring in the non-visualized gallbladders. The chloride concentration shows progressive increase while the bile salt concentration tends to become lower as we pass through Groups III and IV to Group V, until in the latter group there are a number of instances in which no bile salt could be demonstrated. The base concentration decreases, the greatest reduction being found in Group V.

The surgeons' findings and the pathologists' diagnoses in the cases reported in Tables III, IV and V were in all instances in agreement. In Group III the gallbladder was slightly thickened and opaque, may or may not have had adhesions about it and did not contain calculi. The pathologic findings were those of chronic interstitial cholecystitis. The findings in Group IV were similar to those in Group III except for the presence of calculi. In Group V the evidences of cholecystitis were more marked and calculi were always found.

TABLE VI

The time interval from release of an obstruction of the common bile duct to the reappearance of the typical Gregory-Pascoe reaction in liver bile

Patient	Cause of the obstruction	Time interval to reappearance of bile salts
		days
B.	Calculus	16
S.	Calculus	27
J.	Calculus	(16 *)
K.	Calculus	12
D.	Calculus	25
St.	Stricture	15
A.	Stricture (partial)	8
W.	Carcinoma of pancreas	(14 *)
Sch.	Suppurative cholangitis	(15 *)

* Unable to obtain bile after this period; no Gregory-Pascoe reaction in last specimen obtained.

In some cases, as the result of an obstruction of the common bile duct, hepatic secretory suppression had occurred. After the release of the obstruction the bile began draining from the liver and rapidly became what we have reason to believe is normal except for one feature. For a

considerable period, frequently as long as three weeks, the typical color of the Gregory-Pascoe reaction for bile salt was not developed. Instead of the normal blue color, which this method develops, a variety of shades from red to blackish purple were obtained. As the drainage was continued some bile salt made its appearance, but this occurred only late. It would appear that when the liver cells have been damaged during obstruction, the bile salts are for a time excreted either not at all, or at least not in their usual form. In Table VI are given the intervals before reappearance of the typical Gregory-Pascoe reaction for bile salts in a few of these cases.

DISCUSSION

We believe that these analyses of normal human gallbladder bile are the first which have been reported. Hammarsten (6) and others have published data on human bile but the material was either obtained at autopsy or from patients operated on for biliary tract disease, a condition which we have shown greatly alters the anion-cation concentration of gallbladder bile. We are unable to offer any data on normal human liver bile. Obviously drainage of the common duct in a patient with no evidence of a lesion of the biliary tract or pancreas is not justifiable.

We have, however, continued the study of hepatic bile from patients after relief of an obstruction of the common duct, until the concentration of the constituents studied became nearly constant from day to day.

In a previous paper we reported that hepatic bile of the dog is more variable in its electrolyte composition than is serum (1). The same appears to be true for the hepatic bile of man. After long continued drainage of the common duct, so that the hepatic bile was as normal as we had reason to believe it might become, base varied from 151 to 181 m. Eq. per liter. The chloride concentration varied from 76 to 110 m. Eq. per liter, the majority of the figures being below the plasma level for chlorides. This is also true of the hepatic bile of the dog. The calcium concentration of human hepatic bile was found to vary from 1.9 to 10.2 m. Eq. per liter. In the majority of instances the concentration was higher than that of plasma, a relationship which is also true of the dog. The bile salt concentration was even more variable, concentrations from 3.3 to 52.6 m. Eq. per liter being obtained. These data, as a whole, are not unlike those of the dog and although we do not present them as derived from normal human hepatic bile, they are at least indicative of the general trend of its anion-cation concentrations. From these studies we are of the opinion that the hepatic bile of man and dog are similar with respect to the concentrations of the constituents which we are reporting in this paper.

The data on the bile obtained from the normal human gallbladder are quite similar to those from the dog. We have not measured bile pigment or cholesterol in the human cases except in isolated instances, so that at

present we are unable to compare the concentration of these components in dog and human bile. Our few data are not inconsistent with the view that the cholesterol concentration of human bile is higher than that of dog's bile.

It would seem that the more severely damaged the gallbladder the more widely do the concentrations of the constituents under consideration vary from those found in normal gallbladder bile. As long as the gallbladder can be visualized after the administration of sodium tetraiodophenolphthalein, base, calcium and bile salts become concentrated, while chloride is absorbed. As clinical and pathological evidence of change in the gallbladder became more definite the composition of the gallbladder bile approached that characteristic of hepatic bile. In the occasional case (Be. and C., Table V) evidence of some concentration or absorption of the appropriate constituents was found even though the gallbladder was not visualized. The progressive decrease in the bile salt concentration with increasing evidence of damage was the outstanding finding in these studies. Closely paralleling this was the rise in the chloride concentration. Ravdin, Morrison and Smyth (7), Newman (8), and Andrews, Schoenheimer and Hrdina (9) have previously pointed out that the bile salt concentration of the bile from the damaged gallbladder is low. The first authors found that the concentration of bile salt in gallbladder fistula bile in the presence of a diseased gallbladder was lower than the bile salt concentration in common duct fistula bile. Whether the loss of bile salt is due to absorption or change in its structure we are unable to state.

The decrease in the calcium concentration with increasing evidence of gallbladder damage is of interest. No cases in this series were of the type reported by Phemister, Rewbridge and Rudisill (10), where undoubtedly the calcium concentration of the material found in the gallbladder was high. Those cases, however, had complete or incomplete cystic duct obstruction while the studies reported in this paper were made upon subjects with the cystic duct patulous. Whether the low calcium concentration is merely that of hepatic bile or whether it is further lowered due to absorption, precipitation, or dilution is as yet unknown. The rise in the pH and the increase in the total CO_2 concentration of gallbladder bile after gallbladder damage presumably offer favorable conditions for precipitation of calcium. Against the probability of dilution is the observation that in the dog when quantitative studies were made calcium was actually lost from the fluid contents of the gallbladder.

It may well be, however, that the severely damaged gallbladder of man secretes fluid into its lumen as it does in the case of the dog. If this is true the sodium tetraiodophenolphthalein, which after administration is secreted in the liver bile, may be further diluted when it comes to the gallbladder. Thus, what the roentgenologist frequently reports as a

failure of the gallbladder to concentrate, which would imply a static condition, may represent actually a process of active dilution.

The complete absence of bile salt from liver bile obtained shortly after the release of an obstruction which has caused complete hepatic secretory suppression, and its gradual reappearance as the flow of bile continues unimpeded, may be considered as suggestive of the synthesis of bile salts in the liver. The period of time necessary for the reappearance of bile salts, as demonstrated by the typical Gregory-Pascoe reaction, further substantiates the view held by some clinicians that complete recovery of hepatic function is not a rapid process.

Accurate records of the amount of bile obtained daily from a "T" tube in the common duct after the release of an obstruction have been kept in the cases studied. In a subsequent paper we plan to discuss the loss of organic and inorganic salt when the drainage is continued over a long period.

SUMMARY

The concentrations of base, calcium, chloride, and bile salt in the bile from the normal human and dog's gallbladder are reported. The changes in concentration of these substances when the human or dog's gallbladder is diseased are also reported. There appears to be a definite relationship between the extent of the gallbladder damage and the degree to which the activity of the organ is altered from the normal.

Of the constituents which we have studied bile salts and chloride show the more constant changes in disease of the gallbladder. The more severely damaged the gallbladder the lower the bile salt and the higher the chloride concentrations. In the dog, total CO_2 increases in concentration in disease of the gallbladder and presumably this is also true for man.

There is a fairly close correlation between extent of disease, visualization after the administration of sodium tetraiodophenolphthalein and the deviation of the chemical findings in the bile from the normal.

For a number of days after the release of an obstruction of the common bile duct, bile salt, as determined by the typical Gregory-Pascoe reaction, fails to make its appearance. When the liver bile returns to what we suppose to be normal the concentrations of base, calcium, chloride, and bile salt are not unlike those found in the normal liver bile of the dog.

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