II. BILE CHOLESTEROL

Fluctuations Due to Diet Factors, Bile Salt, Liver Injury and Hemolysis

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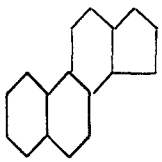
The blood and body fluids are so crowded with "chemical messengers" and vitamines that to some readers it appears a miracle that these substances ever reach their destination. Cholesterol has been looked upon as an innocent bystander, inert and going along with the crowd. Some of the recent work with hormones and vitamines would seem to focus attention on cholesterol as a close relative of other sterols and perhaps of ergosterol and the group of fat soluble vitamines. Further work with hormones (estrin and the male hormone) indicates a chemical constitution relating these "messengers" to the sterols. The same four ring nucleus is common to all these substances (18)



Therefore instead of an innocent bystander cholesterol may prove to be a messenger of importance and authority related to many vital body processes.

It can be seen from the tables below that cholesterol is influenced profoundly by bile salt metabolism and circulation. Bile salt feeding together with cholesterol may give maximal values for cholesterol in the bile. All evidence (15) points to the liver cell as the only source of bile salts but this does not necessarily mean that cholesterol is produced in the liver cell. However, it would be difficult indeed to prove

that the liver is not concerned with cholesterol metabolism and its production in the body.

It is significant that the blood plasma of the dog contains 10 to 20 times as much cholesterol per 100 cc. as does the bile. Cholesterol in blood plasma averages 150–300 mg. per 100 cc. in contrast to bile which averages 10–15 mg. per 24 hour output in a total volume of 80–130 cc. This suggests a liver threshold of elimination but if such a threshold does exist it differs conspicuously from the renal threshold as it is understood today. It is possible to raise the blood cholesterol without a large increase in bile cholesterol and also to increase the cholesterol elimination in the bile without a change in blood cholesterol concentration. Cholesterol esters make up a large part of the blood cholesterol but the esters do not appear in the bile under the conditions of these experiments. The normal liver cell if it has a threshold for free cholesterol will not pass on into the bile any cholesterol esters. This question is receiving further study.

It may be argued that cholesterol as it appears in the bile is dependent upon the circulation of the bile salts. This may be in part a physical relationship as bile salts increase the solubility of cholesterol in the whole bile. It is also possible that the bile salts exert an influence upon the liver cell, modifying its physiological state and permitting the passage of cholesterol. It is generally accepted that the bile salts modify definite body functions in the gastro-intestinal tract in the external sector of their cycle in the body. We believe that the *internal sector of the bile salt cycle* may be even more important for the body, and that the hepatic cells and other body cells may be modified in their activity by the presence of bile salts. Interesting types of intoxication which develop in the fistula dog after long periods of bile salt deprivation point in this direction.

There is no dearth of experimental observations dealing with bile cholesterol in humans and animals. McMaster (9) has reviewed the earlier work and points out that much of the recorded data was unsatisfactory because of the type of bile fistula used. He showed that cholesterol in the bile can be increased by diets rich in cholesterol. The bile fistula introduced by Rous and McMaster (10) enables the investigator to collect accurately the 24-hour sample of sterile bile and marked a distinct advance in this field of study. Methods for bile cholesterol analysis have been unsatisfactory and inaccurate until quite recently and the recorded data are therefore inaccurate and subject to review. In the method used by McMaster

(9) the bile pigments introduce large errors and his base line for bile cholesterol output in the dog runs about double the amount recorded in the tables below.

Biliary cholesterol has been studied by D'Amato (2), Stepp (17), Dostal and Andrews (3), Fox (5), Salomon and Silva (16), Gardner and Fox (6), Elman and Taussig (4), McClure (8) and many others. Some of these papers deal with human, others with animal bile. The objections noted above apply to these observations. The greatest diversity of opinion on all phases of the subject is revealed by these papers.

Methods

The methods used in the quantitative determination of bile cholesterol are described above (Paper I). The bile fistula dogs were prepared according to the method of Rous and McMaster (10). Meticulous attention to the details of aseptic technique is needful in the care and daily bile collection in these animals (12). Their general supervision requires the bulk of the time of one technician. This type of fistula is made with excision of the gall bladder and insertion of a cannula in the common bile duct so that the bile is collected in a sterile bag. A comfortable canvas binder retains the bag and enables the dog to live a quiet and comfortable life for many months. It is highly important that these dogs remain in excellent clinical condition with little or no loss of weight and freedom from gastro-intestinal disturbances. Little significance can be attached to observations on dogs showing clinical abnormalities which are usually recorded in the published experiments from many laboratories.

The standard or control diet consists of a bread prepared in the laboratory and much used in the anemia colony. The bread contains wheat flour, starch, bran, sugar, cod liver oil, canned tomatoes, canned salmon, yeast and a salt mixture. Its preparation has been adequately described (20). This is a complete diet for the normal dog and will maintain anemic animals in health indefinitely. The *control periods* given in the tables below *precede* immediately the periods dealing with special diets, liver injury, bile feeding, etc. After operation there may be fluctuations in bile cholesterol which may be due to obscure factors. For this reason the dog was observed for a period of 7–10 days before the regular control periods were begun.

EXPERIMENTAL OBSERVATIONS

Brief clinical histories of the several bile fistula dogs are given in the following paragraphs. It will be noted that the weight at the end of the experimental observations is in no instance lower than the weight recorded at the beginning. This means excellent clinical condition, good food consumption and no gastro-intestinal disturbances. Fasting or intoxication will always reduce the normal output level of bile cholesterol.

Clinical Histories

Dog 32-161. Adult female white bull mongrel, operation Jan. 12, 1933. Weight at beginning of analyses 14.4 kg. Hemoglobin 144 per cent. This animal's weight remained constant except during a period of liver injury due to intravenous injection of hematin (Table 27) when the weight dropped to 13.0 kg. This loss was rapidly recovered and at present (Oct. 6, 1933), hemoglobin 111 per cent, the animal weighs 14.8 kg. The hemoglobin level has maintained a similar constant level with the exception of periods during which it has been lowered as a result of direct experimentation. Food consumption has always been good. The animal has been in excellent physical condition throughout the period of observation.

Dog 31-27. Adult female long haired mongrel, operation May 1, 1932. Weight 13.2 kg. Hemoglobin 119 per cent. Weight and hemoglobin have shown slight variation. Food consumption excellent. The animal has been exceedingly lively. On May 29, 1932, bile became infected and dog killed under anesthesia. Weight 13.7 kg., hemoglobin 105 per cent.

Dog 31-331. Adult female hound mongrel, operation June 1, 1932. Weight 16.9 kg. Hemoglobin 132 per cent. The hemoglobin showed slight variation and there was slight gain in weight to 17.4 kg. The animal was lively and consumed all of its food. On Dec. 21, 1932, the bile became infected and the animal was killed under anesthesia. At this time the weight was 17.4 kg. and the hemoglobin 105 per cent.

Dog 31-203. Adult female setter mongrel, operation Jan. 26, 1932. Weight 15.0 kg. Hemoglobin 107 per cent. The weight increased somewhat over a long period of experimentation, rising as high as 17.5 kg. The animal was in excellent physical condition throughout and consumed its food completely. The hemoglobin varied somewhat, going as low as 67 per cent during some of the experimental periods. The bile became infected June 3, 1933, and the animal was subsequently killed under anesthesia. At this time the animal weighed 15.9 kg., hemoglobin 93 per cent.

Table 21 gives characteristic control observations on two bile fistula dogs. Dog 32-161 shows a normal level in the first control period but a low normal in the second control period which was 7 months subsequently and followed a period of liver injury (see Table 27). The fore periods on salmon bread immediately preceded the test periods on calves' brains. In the control periods the fluctuations in bile cholesterol output from day to day rarely exceeds 1–2 mg. (see Table 25).

Calves' brains in the older experiments reported in the literature were usually fed with egg yolk and assumed to be in part responsible for the bile cholesterol increase if any was observed. In our experi-

ments the calves' brains alone (containing approximately 1.5 gm. cholesterol) have a negligible effect as a 10 per cent increase is within physiological fluctuations related to uncontrollable factors. The significant rise in bile cholesterol output when bile salt is added will be discussed under Table 23.

We know of no satisfactory explanation for the observations (Table 21) that the feeding of cholesterol in calves' brains gives no increase in biliary cholesterol, whereas the egg yolk feeding will give a definite increase (Table 23). It has been suggested (11) that the presence of the phosphatides and cerebrosides may prevent the absorption of the brain cholesterol.

TABLE 21

Bile Cholesterol and Calves' Brains Feeding

Control diet—salmon bread				Control diet + calves' brain 230 gm. daily				
Dog No.	Duration	Bile volume daily average	Cholesterol average daily output	Duration	Bile salt fed	Bile volume daily average	Cholesterol average daily output	Cholesterol increase
	days	*cc.*	*mg.*	*days*	*gm.*	*cc.*	*mg.*	*per cent*
32-161	7	129	12.6	4	0	130	14.3	15
32-161	6	86	7.9	8	3	145	17.2	118
31-331	3	115	10.0	4	0	133	10.8	8
31-331	7	125	12.8	4	0	146	13.1	10

Table 22 shows some satisfactory and representative experiments with widely different food factors all done on the same dog which was in perfect physical condition and ate all the food as indicated. The sugar diet and zein (digested with trypsin) were given daily by stomach tube.

The control salmon bread diet periods show a large bile elimination—an average of about 140 cc. daily, and a uniform output of bile salt—an average of about 1.1 gm. per day.

Liver added to this control diet causes little or no change in bile volume, cholesterol or bile salt output. *Lean beef* feeding causes a distinct rise in bile salt but not in the cholesterol output. *Sugar* alone fed to a bile fistula dog always causes a sharp drop in bile volume and bile salt output. The drop in bile cholesterol is less conspicuous.

TABLE 22

Bile Cholesterol and Food Factors

Dog 31-203.

Control diet—salmon bread					Animal and grain protein and sugar				
Duration	Weight	Bile volume daily average	Bile salt daily average	Cholesterol average daily output	Duration	Diet—daily	Bile volume daily average	Bile salt daily average	Cholesterol average daily output
days	*kg.*	*cc.*	*mg.*	*mg.*	*days*		*cc.*	*mg.*	*mg.*
11	16.3	120	1078	14.1	14	Salmon bread + pig liver 300 gm.	109	1189	14.2
8	15.7	149	1228	12.0	6	Beef cooked 680 gm.	134	1510	13.0
9	16.0	154	1142	13.5	4	Sugar 50 gm.	67	523	8.3
9	17.3	139	1219	14.9	3	Zein 50 gm.	91	501	5.4

TABLE 23

Bile Cholesterol Influenced by Egg Yolk and Bile Salt Feeding

Control diet—salmon bread					Control diet + egg yolk, bile salt or bile daily					
Dog No.	Weight	Duration	Bile volume daily average	Cholesterol average daily output	Duration	Yolks daily	Bile salt daily	Bile volume daily average	Cholesterol average daily output	Cholesterol increase
	kg.	*days*	*cc.*	*mg.*	*days*		*gm.*	*cc.*	*mg.*	*per cent*
31-27	13.4	7	110	10.5	6	4	0	109	15.0	50
31-27	13.3	6	123	10.3						
31-331	17.0	6	132	13.5	3	6	0	148	18.6	39
31-331	16.4	5	129	12.5	3	6	1*	171	19.8	46
31-331	16.5	8	111	10.9	4	0	1*	140	16.8	54
31-331	17.0	23	104	10.3	10	5	1*	194	21.5	105
31-203	16.9	4	153	13.3	4	4	0	162	17.5	32
32-161	14.2	6	108	8.2	8	0	1	124	12.0	47
32-161	14.4	3	108	8.8	9	0	1*	132	15.7	79
32-161	14.2	6	108	8.6	9	0	3	159	19.3	124
32-161	14.6	3	86	8.8	10	3	3	127	18.6	111

* This amount of bile salt given as whole bile which contains approximately 10 mg. cholesterol.

Zein is an incomplete protein which we have used in a study of bile salt metabolism. It causes a sharp fall in bile volume and bile salt output and even more conspicuous drop in bile cholesterol. This deserves further study.

At any rate we see that it is possible to dissociate bile volume, bile salt and bile cholesterol concentration. In a general way the bile cholesterol-bile salt ratio is about 1 to 100 but this is not constant.

The gist of Table 23 is that egg yolk feeding without bile or bile salt will cause a 40–50 per cent increase of bile cholesterol. A single egg yolk contains 0.3–0.5 gm. cholesterol. Bile alone by mouth containing 1 gm. bile salt will cause about the same increase in bile cholesterol. When larger doses of bile salt (3 gm.) alone are fed we note an increase of over 100 per cent of bile cholesterol and there is no further rise in bile cholesterol if we give this dose of bile salt plus egg yolks. This point has not been observed by other workers and gives less emphasis to heavy cholesterol feeding (egg yolks). It is of interest that *blood cholesterol* remains unchanged with bile salt feeding but rises to high levels when bile salt plus egg yolk is fed. The bile cholesterol elimination remains at the same level in both experiments (Dog 32-161, Table 23). Under normal physiological conditions with an intact bile circulation and no bile fistula it is probable that heavy cholesterol feeding would cause no reaction (Table 23) or at best a slight rise in bile cholesterol (see Table 24).

Table 24 indicates the *maximum level* to which we have been able to push cholesterol excretion in the bile by means of continued bile feeding plus egg yolk plus large supplementary bile salt additions. This dog was in perfect physical condition and consumed daily its salmon bread ration. The supplements added to this ration or given by stomach tube are shown (Table 24). For 13 days preceding the 1st day given in Table 24, the dog was refed daily the total bile output as collected, minus 10 cc. for routine analysis. It has been shown elsewhere (21) that refeeding of bile over considerable periods will raise the bile salt output to a level which is sustained at about 7–8 gm. bile salt output per 24 hours. This dog had not reached this plateau at the time the observations were begun in Table 24 and we note a bile salt output of 4.5 gm. per day. Meanwhile the bile cholesterol has increased slowly from the control level at the start of bile refeeding—

9.3 mg. to 21.5 mg. When bile salt (3 gm.) is added to the bile refeeding we note a great increase in bile cholesterol—42.6 mg. per 24 hours. The peak of cholesterol production follows by 1 day the peak of bile salt output. Egg yolks added to the bile refeeding increase the

TABLE 24

Bile Cholesterol Influenced by Cholesterol and Bile Salt Feeding

Dog 32-161.

Date	Diet—salmon bread daily	Bile volume daily output	Cholesterol daily output	Bile salt daily output
		cc.	*mg.*	*gm.*
Oct. 10	Bile 178 cc.	198	21.5	4.51
Oct. 11	Bile 198 cc.	218	21.6	4.22
Oct. 12	Bile 220 cc., bile salt 3 gm.	230	16.5	
Oct. 13	Bile 272 cc., bile salt 3 gm.	282	21.0	
Oct. 14	Bile 290 cc., bile salt 3 gm.	300	24.0	
Oct. 15	Bile 286 cc.	308	38.5	8.66
Oct. 16	Bile 300 cc.	332	42.6	6.83
Oct. 17	Bile 246 cc.	256	27.6	
Oct. 18	Bile 228 cc. + 4 egg yolks	238	30.2	
Oct. 19	Bile 302 cc. + 4 egg yolks	312	36.3	
Oct. 20	Bile 255 cc. + 4 egg yolks	265	34.4	
Oct. 21	Bile 301 cc. + 4 egg yolks	332	30.2	6.57
Oct. 22	Bile 253 cc.	273	30.4	5.10
Oct. 23	Bile 234 cc.	244	39.4	
Oct. 24	Bile 218 cc.	228	28.5	
Oct. 25	Bile 262 cc.	272	28.0	
Oct. 26	Bile 248 cc. + bile salt 3 gm. + 4 egg yolks	258	27.2	
Oct. 27	Bile 334 cc. + bile salt 3 gm. + 4 egg yolks	344	34.4	
Oct. 28	Bile 294 cc. + bile salt 3 gm. + 4 egg yolks	304	46.1	
Oct. 29	Bile 364 cc. + bile salt 3 gm. + 4 egg yolks	386	48.3	9.47
Oct. 30	Bile 346 cc.	378	61.0	9.84
Oct. 31	Bile 282 cc.	292	52.2	
Nov. 1	No bile	325	46.8	
Nov. 2	No bile	152	9.1	

bile cholesterol almost as much as does the bile salt but meanwhile the bile salt output is on the decline.

Maximum figures for bile cholesterol (61 mg. per 24 hours) are observed when we combine egg yolk and bile salt with the whole bile refeeding. This high level is more than 6 times the base line but

if we consider as normal the output due to bile refeeding then the output is doubled by egg yolk and bile salt supplementary feeding (Table 24).

When bile refeeding is stopped the output falls promptly to the control level on salmon bread diet—9.1 mg. cholesterol per 24 hours. The dog was then fasted for 2 days and the cholesterol fell to 4.3 mg.

Table 25 shows that isatin by mouth or decholin by vein or by mouth will give a definite cholagogue effect without any influence on cholesterol elimination by the bile. In fact as these substances cause some

TABLE 25

Isatin and Decholin Show Cholagogue Effect but Negative Influence on Bile Cholesterol

Control diet					Control diet + isatin or decholin daily				
Dog No.	Duration	Bile volume daily average	Cholesterol average daily output	Bile salt daily average	Duration	Bile volume daily average	Isatin or decholin	Bile salt daily average	Cholesterol average daily output
	days	*cc.*	*mg.*	*mg.*	*days*	*cc.*		*mg.*	*mg.*
31-331	8	115	8.8		3	155	Isatin—5 gm.		8.0
31-161	10	87	8.3		6	135	Isatin—5 gm.		5.6
31-161	2	90	9.1	1174	2	120	Decholin*	1504	8.4
31-161	3	125	8.8	1100	4	130	Decholin*	1211	3.0
31-161	10	87	8.3	1100	4	238	Decholin—3 gm.	1670	6.6

* Decholin given by vein daily—2 gm.

gastro-intestinal disturbance and occasional vomiting we note more or less decrease in cholesterol elimination. Decholin by vein in one instance caused a good deal of clinical disturbance, very low food consumption and a very low cholesterol output (3.0 mg. per 24 hours). This is practically the fasting level.

It is known that isatin (14) causes no increase in bile salts but decholin does cause a moderate increase in bile salt elimination. This does not compare with the reaction to bile salt by mouth which is subsequently eliminated within 24 hours in amount practically 100 per cent of the intake. We cannot say whether the decholin may be

eliminated as such in the bile as the method used would not detect it. Evidently some of the introduced decholin is linked in the body with taurin to yield taurocholic acid. The cholagogue reaction to decholin is more conspicuous when the drug is given by mouth as compared with intravenous administration. The last two figures for bile salts (1100 mg.) in control periods (Table 25) are general average values.

Table 26 shows a satisfactory experiment in which moderate liver injury was produced by small doses of chloroform by mouth. The

TABLE 26

Bile Cholesterol with Liver Injury and Repair

Dog 32-161.

Date	Diet	Weight	Bile volume daily output	Cholesterol daily output
		kg.	*cc*	*mg.*
Aug. 14	Salmon bread	14.5	90	9.4
Aug. 15	Salmon bread		76	8.1
Aug. 16	Salmon bread		132	8.2
Aug. 17	Salmon bread		108	8.5
Aug. 18	Salmon bread		80	8.2
Aug. 19	Salmon bread		82	7.3
Aug. 20	Salmon bread	14.5	84	9.3
Aug. 21	Salmon bread + chloroform 3 cc.		92	8.2
Aug. 22	Salmon bread + chloroform 3 cc.		50	4.9
Aug. 23	Salmon bread + 100 gm. karo syrup		18	1.6
Aug. 24	Salmon bread + 100 gm. karo syrup	14.6	10	0.4
Aug. 25	Salmon bread + 100 gm. karo syrup		26	1.3
Aug. 26	Salmon bread		42	2.8
Aug. 27	Salmon bread		70	5.4
Aug. 28	Salmon bread	14.5	74	6.0

repair took place promptly and was probably complete in 7–10 days. There was no clinical disturbance, the dog acting normally and eating all food. Bile volume shows a sharp fall to about 10 per cent of normal and the bile cholesterol falls even closer to zero. Bilirubinemia developed with an icterus index of 10 and bile was present in the urine. From published experiments (22) we know that the bile salts in the bile also fell very close to zero. The return toward normal in bile cholesterol is well shown (Table 26) and parallels closely the liver repair and bile salt excretion curve (22) as given elsewhere. From other

experiments we know that in such animals the signs of liver injury are very slight as shown by histological study—a few cells about the central vein showing fat or hyaline necroses. The repair is prompt and completed usually within 7 days.

Table 27 shows a severe liver injury followed by slow return to normal over a period of 4–5 weeks. In connection with other experiments this dog was given hematin intravenously which caused severe and almost fatal poisoning. From autopsy examinations in other dogs we

TABLE 27

Bile Cholesterol with Severe Liver Injury and Slow Repair

Dog 32-161.

Date	Diet	Weight	Bile volume daily output	Cholesterol daily output
		kg.	*cc.*	*mg.*
Feb. 18	Salmon bread	14.3	124	11.5
Feb. 19	Salmon bread		106	11.5
Feb. 20	Salmon bread + 0.2 gm. hematin by vein		110	11.0
Feb. 21	Salmon bread + 0.2 gm. hematin by vein		58	6.5
Feb. 22	Salmon bread + 20 gm. glucose by vein		No bile	—
Feb. 23	Salmon bread		No bile	—
Feb. 24	Salmon bread + * 50 cc. bile by mouth		No bile	—
Feb. 25	Salmon bread + * 50 cc. bile by mouth	13.0	30	—
Feb. 26	Salmon bread + * 50 cc. bile by mouth		44	2.9
Feb. 27	Salmon bread		96	4.6
Feb. 28	Salmon bread	13.6	104	4.7
Mar. 23	Salmon bread	14.2	62	6.1
Apr. 3	Salmon bread	14.0	82	9.3
Apr. 17	Salmon bread	14.0	122	10.3

* This bile contained approximately 5 mg. cholesterol + 0.6 gm. bile salts.

have assurance that there resulted an extensive central liver necrosis, which healed slowly. This dog was severely intoxicated and appeared clinically very sick. Bilirubinemia was severe and the blood fibrinogen fell to 170 mg. per cent. There was bleeding from vein punctures. Clinical improvement began 4 days after the second hematin injection but recovery was slow. There was some loss of weight. For 3 days there was complete suppression of bile flow. The bile cholesterol values came back slowly. In these severe injuries the change of cho-

lesterol output is less spectacular than with slight injuries when the bile flow is not suppressed. These data are in accord with those presented in Table 26.

Blood Destruction and Cholesterol Elimination in Bile

It has been claimed by some investigators and assumed by many others that red cell destruction sets free the cholesterol in the red cell matrix, which logically might well appear in the bile. Other materials coming from red cell destruction (pigments and iron) appear in the liver or bile so why not cholesterol? But experiments indicate that this is not the way of body physiology.

The experiment outlined just below shows no increase in bile cholesterol but rather a slight decrease, probably due to slight intoxication by the hydrazine used to destroy red cells.

Dog 32–161 (see clinical history above). Weight 14 kg., hemoglobin 158 per cent, normal in all respects. The fore period of 10 days showed a somewhat low normal cholesterol daily output of 6.3 mg. During a 4 day period the dog was given subcutaneously 100 mg. daily of acetylphenylhydrazine. This caused a drop in the hemoglobin level to 86 per cent. Calculating the destroyed hemoglobin on the basis of the dog's weight and our general experience with anemia in dogs, it is safe to say that not less than 100 gm. hemoglobin were destroyed. If any cholesterol is to be derived from hemoglobin destruction and appear in the bile, this would seem an adequate test. During the 4-day period of hydrazine administration and the subsequent 10 days, the bile cholesterol averaged 5.5 mg. per day. The after period of 16 days shows a bile cholesterol daily output of 7.6 mg. At the end of this last period the hemoglobin level had come back to 112 per cent. The dog was fed the standard control salmon bread diet throughout and the weight was unchanged.

DISCUSSION

Possibly clinical treatment of abnormalities of the biliary system has not taken into consideration some of the facts established by experimental study of the bile. This may not be the place for a discussion of clinical problems but it may be proper to indicate that certain cholagogues can be used with advantage in human cases presenting irritation or inflammation of the biliary tree. Under these conditions it is recognized that stasis of bile and high cholesterol concentration may favor the precipitation of cholesterol with subsequent building

up of gall stones. It is logical to assume that on such occasions an active flushing of the biliary ducts by means of some cholagogue might forestall the unfortunate precipitation of debris and cholesterol. Also bile salts in addition to their active cholagogue effect will appear in the bile and help to hold any excess of cholesterol in solution. It is even conceivable that a small soft precipitate of cholesterol under such conditions might go back into solution, as bile salts effect rapid solution of cholesterol. In the dog's gall bladder it has been shown (1, 7) that human gall stones will be dissolved during the course of many weeks.

The cholesterol-bile salt ratio is about 1 to 100 in the bile fistula bile but considerable variations may be noted. The ratio in the blood must be vastly different although we cannot say how much bile salt is to be found in the circulating blood. As the normal blood plasma contains about 200 mg. cholesterol per 100 cc., if the same ratio obtained we should find about 20 gm. bile salt per 100 cc. plasma which is ridiculous. It is probable that the blood plasma contains only a few milligrams of bile salt per 100 cc. but present methods do not permit us to measure this with any accuracy.

Therefore we have a considerable amount of cholesterol in circulation—for example a 10 kg. dog would have a plasma volume of 500 cc. and a cholesterol concentration of 150 to 300 mg. per cent—or 750 to 1500 mg. in circulation. From this reservoir of ± 1 gm. plasma cholesterol we have only a trickle of 10–20 mg. per day appearing in the bile. Meanwhile the feeding of cholesterol and bile salt may change the level of the plasma reservoir of cholesterol by large amounts. All this would point to the bile cholesterol elimination as a minor shunt for certain surplus material. We do not accept this conclusion without protest and believe that the bile cholesterol is related in some way to the important internal cholesterol metabolism which goes on in the liver cell.

Because cholesterol and bile salt have marked similarities in their structural formulas—both contain the same four ring nucleus—it has been claimed that cholesterol is the precursor of bile salt. This thesis has been shown by Smith and Whipple (13) to be untenable. But may a surplus of bile salt be changed to cholesterol? This seems to be unlikely on theoretical grounds and there is no real support for this

hypothesis on experimental grounds. The body seems able to dispose of any surplus of bile salts without any demonstrable increase in cholesterol stores or elimination. However the experimental data are not adequate as yet to exclude this possibility.

SUMMARY

Under uniform diet conditions the normal bile fistula dog will eliminate pretty constant amounts of cholesterol—about 0.5 to 1.0 mg. cholesterol per kilo per 24 hours.

Diets rich in cholesterol (egg yolk) will raise the cholesterol output in the bile but compared to the diet intake (1.5 gm. cholesterol) the output increase in the bile is trivial (5–15 mg.). Calves' brains in the diet are inert.

Bile salt alone will raise the cholesterol output in the bile as much and often more than a cholesterol rich diet.

Bile salt plus egg yolk plus whole bile give maximal output figures for bile cholesterol—60 mg. per 24 hours.

Liver injury (chloroform) decreases both bile salt and cholesterol elimination in the bile.

Blood destruction (hydrazine) fails to increase the bile cholesterol output and this eliminates the red cell stroma as an important contributing factor.

Certain cholagogues (isatin and decholin) will increase the bile flow but cause no change in cholesterol elimination.

The ratio of cholesterol to bile salt in the bile normally is about 1 to 100 but the bile salts are more labile in their fluctuations.

The ratio is about reversed in the circulating blood plasma where the cholesterol is high (150–300 mg. per cent) and the bile salt concentration very low.

Cholesterol runs so closely parallel to bile salt in the bile that one may feel confident of a physical relationship. In addition there is a suspicion that the bile cholesterol is in some obscure fashion linked with the physiological activity of hepatic epithelium.

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