III. BLOOD PLASMA CHOLESTEROL

Fluctuations Due to Liver Injury and Bile Duct Obstruction

By WILLIAM B. HAWKINS, M.D., and ANGUS WRIGHT, M.D.

(*From the Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, N. Y.*)

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As the fluctuations of the bile cholesterol were followed in bile fistula dogs, Paper II, changes were observed which suggested that a study of *blood plasma cholesterol* might yield information of value. Under varying conditions the fluctuations in bile and blood plasma cholesterol might be correlated and lead to a better understanding of cholesterol metabolism. Much work has been done to indicate the changes in blood plasma cholesterol which might be of significance in clinical diagnosis of human disease. This is referred to below in a brief review of this literature.

It is notorious that human disease presents a very complex mixture of abnormal functions, and consequently it is at times extremely difficult to evaluate data derived from study of human material. Little experimental work has been done with animals to produce one single type of injury or abnormal condition and observe the alterations in blood plasma cholesterol which might follow. From this study one is forced to conclude that like many other tests for liver function or liver disease the blood plasma cholesterol may give information of some value but diseased conditions may be present without significant disturbance of total blood cholesterol or of the esterified cholesterol ratio.

A drop in the ratio of cholesterol esters to total cholesterol of the blood plasma was first observed by Feigl (7) in cases of acute yellow atrophy. This phenomenon was more extensively investigated by Thannhauser and Schaber (15) who explained this change on the basis of injury of the liver with consequent impairment of function of an enzyme of the liver cells which is effective in hydrolyzing esterified cholesterol. Thannhauser (14) has demonstrated this enzyme and its action in bile. Gardner and Gainsborough (8) interpreted the low cholesterol ester values in pathological states of the liver as being due to failure of absorption of cholesterol

as a result of lack of bile in the intestine, or as an alternative hypothesis, that in the absence of fat intake from the intestine, the body utilizes the fatty acid already combined with cholesterol—a de-esterification. Epstein (6) has noted hypocholesteremia and dissociation of the normal ratio of esterified to total cholesterol in patients with parenchymatous liver disease. He has also commented on hypercholesteremia occurring in certain cases of biliary obstruction. Mjassnikow (12) reports hypocholesteremia in liver injury caused by phosphorus and arsphenamine. His experimental animals were dogs and rabbits.

Experimental Methods

We have used the colorimetric method of Bloor (1) and Bloor and Knudson (3) for the determination of blood plasma cholesterol, with minor modifications described in Paper I. It is worth while to note here again that in a long series of comparisons of the colorimetric and digitonin methods for the determination of blood plasma cholesterol, Bloor (2) has found the colorimetric method to run consistently about 20 per cent higher than the digitonin method, and expresses the opinion that the colorimetric value more closely approximates the actual. This is in accord with observations made by one of us (Paper I).

The dogs are bled every morning at the same time and fed in the early afternoon each day, so that the blood samples are free from the questionable influence (9, 4) of alimentary absorption. Approximately 10 cc. of blood drawn from the jugular vein is received into a 15 cc. calibrated hematocrit tube containing 2 cc. of a solution of 1.4 per cent sodium oxalate and centrifugalized for 35 minutes at a speed of 2600 R.P.M. The same hematocrit tube is used for the same animal each day.

Determinations of the icterus index are made by the method described by Cutten *et al.* (5) and the values recorded are in milligrams of bilirubin per liter of plasma. The method of Jones and Smith (10) is used in the determination of fibrinogen.

Parenchymatous liver injury is produced by giving small doses of chloroform by mouth. The chloroform is suspended in a slightly viscous solution of starch or dissolved in small quantities of cotton seed oil and given by stomach tube. The chloroform dissolved in cotton seed oil is better tolerated.

EXPERIMENTAL OBSERVATIONS

The effect of parenchymatous liver injury upon the blood plasma cholesterol was studied in ten experiments performed on nine dogs, in some as uncomplicated parenchymatous injury and in others associated with biliary obstruction. The results of the simple parenchymatous injury experiments are uniform (see Chart A). After an adequate control period the animals are given small daily doses of chloroform by mouth in a starch solution or in cotton seed oil. There results a progressively increasing jaundice and after varying lengths

of time the blood plasma cholesterol values begin to drop. At the peak of liver injury, as indicated by marked jaundice and intoxication, the ratio of esterified cholesterol to the total cholesterol decreases to 30 per cent or less (normal ratio 40–70 per cent). If the dogs are allowed to recover, the ester ratio and the values for free and esterified cholesterol mount rapidly with subsidence of the jaundice. Sometimes recovery was attended by slightly higher plasma cholesterol values than noted in the control periods.

TABLE 31

Blood Plasma Cholesterol—Chronic Chloroform Liver Injury

Dog 32-143.

Date	Weight	Food consumed	Icterus index	$CHCl_3$ by mouth daily	Total cholesterol	Esters, cholesterol	Ester ratio
	kg.	*per cent*		*cc.*	*mg. per cent*	*mg. per cent*	*per cent*
Mar. 26–Mar. 30	20.7	100	0	0	155	89	58
Mar. 31–Apr. 8	21.3	100	14	4	163	88	54
Apr. 9–Apr. 17	21.0	100	25	4	145	81	55
Apr. 18–Apr. 26	19.5	100	25	4	119	59	48
Apr. 27–May 5	19.5	100	32	9	147	75	51
May 6–May 17	18.8	80	23	15	131	64	48
May 18–May 23	17.6	50	38	15	124	60	48
May 24		25	53	20	131	49	37
May 25		50	58	20	106	48	45
May 26		14	48	20	96	32	33
May 27	17.6	0	50	20	89	25	28
May 28		0	62	20	89	30	34
May 29		0	40	20	106	27	25
May 30		0	60	20	120	37	31
May 31		0	60	20	190	28	15

In all cases when the ratio of esterified cholesterol dropped to 30 per cent or less of the total cholesterol the animals were critically ill. The ratio of esterified cholesterol taken just before death varied from 0 per cent to 26 per cent of the total.

Table 31 (Dog 32-143) shows the effect upon the blood plasma cholesterol of parenchymatous liver injury caused by repeated daily doses of chloroform by mouth.

Previous to this experiment this dog had been subjected to 1 hour of chloroform anesthesia (see Table 32) and to a course of chloroform by mouth with development of moderately severe parenchymatous injury.

A most interesting finding in this experiment was the large amounts of chloroform which were tolerated over a long period of time. Over a 2-month period this animal was given over 12 times the amount of chloroform necessary to injure severely the liver of a normal animal over a 2-week period. MacNider (11) has reported that liver cells may acquire resistance to chloroform and uranium after previous injury of the liver by these poisons.

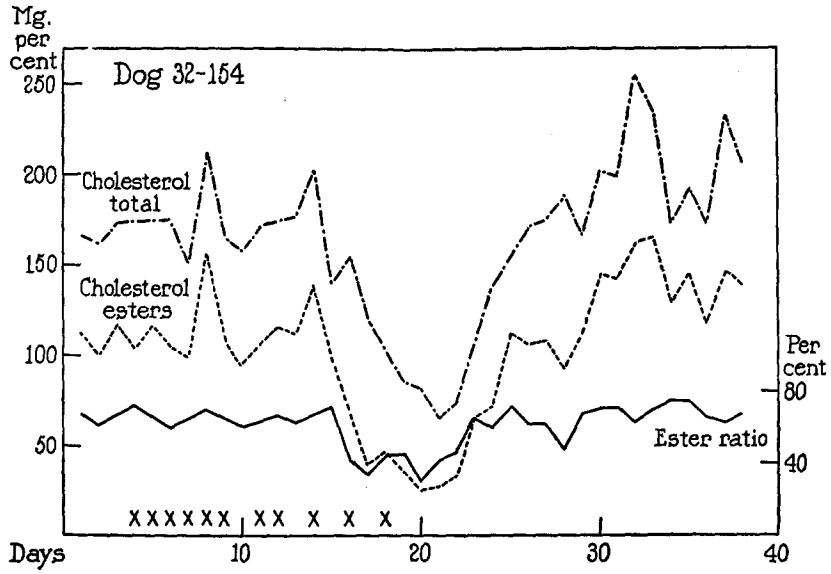

CHART A. Blood plasma cholesterol—chloroform liver injury.
× = 3 cc. chloroform by mouth.

In the table the first seven horizontal lines indicate average values for the periods noted. In the period from Apr. 18 to Apr. 26 the dog was fasted for 5 consecutive days during which the chloroform was continued. In spite of fasting the ratio of esterified to total cholesterol remained normal, 48 per cent.

For 50 days the dog was given chloroform by mouth in amounts as indicated, and during this period there was persistent jaundice but no significant change in the blood plasma cholesterol. In the last 8 days of the experiment the jaundice became more marked. The animal showed definite changes in the blood plasma cholesterol, with rapid physical decline, and drop in the ratio of esterified cholesterol to 15 per cent of the total on the day of death. During the latter part of the experiment the animal left food and in the last 5 days did not eat.

Autopsy revealed generalized jaundice. The extra-hepatic biliary ducts were patent and there was bile in the duodenum. The liver, grossly, was firm, uniformly yellow in color with no alteration of the lobulation. Histologically the liver showed severe fatty degeneration with normal appearing liver cells only in the portal regions. There was also a terminal pneumonia. Other organs were normal.

Chart A, Dog 32-154, illustrates a similar experiment in which the dog developed pronounced liver injury within a period of 16 days. Eleven doses of chloroform 3 cc. were given. On the 15th day of the experiment there was significant change in the blood plasma cholesterol. As hypocholesteremia developed, the ratio of esterified to total cholesterol began to drop and continued to fall until on the 20th day it was 30 per cent of the total. At this point the dog was severely intoxicated and jaundice was marked. Sugar was given by vein during the day with consequent betterment of the animal's condition. Recovery as indicated by rise in the blood plasma cholesterol and decrease of bilirubinemia was rapid. The ratio of esterified to total cholesterol returns to normal before the control levels of total cholesterol values are reached.

Two more dogs, 31-169 and 32-172, gave similar results under the same experimental conditions. Dog 31-169 died at the height of liver injury and autopsy showed generalized jaundice and a fatty liver. There was a duodenal ulcer. Histologically in the liver there was fatty degeneration of practically all liver cells.

The results of acute liver damage caused by 1 hour of chloroform anesthesia are given in Table 32, Dog 32-143. In the days following anesthesia the dog was intoxicated and showed marked bilirubinemia. Surprisingly enough in the face of the results recorded above the blood plasma cholesterol did not vary beyond the usual diurnal range.

Dog 32-265 was fasted for 48 hours before chloroform anesthesia of 1 hour duration. Following this, jaundice was marked but again blood plasma cholesterol values remained normal. The dog was killed under ether anesthesia 48 hours after the chloroform was administered. Autopsy showed moderate generalized icterus. There was definite diminution of fibrinogen. The liver showed grossly central necrosis and histologically there was hyaline necrosis involving about half of the liver lobule with a peripheral zone of fatty cells. In the portal regions normal appearing liver cells persisted.

The effect of *biliary obstruction* upon the blood plasma cholesterol was studied on six dogs. Four of these animals were obstructed surgically after a period of control observation, the others were bile fistula

dogs of the type developed by Rous and McMaster (13) which had previously been studied in connection with bile and blood plasma cholesterol prior to obstruction. All of these animals were completely obstructed as evidenced by absence of bile pigments in the feces, and marked cholemia. At autopsy careful investigation of the biliary tract was made to check the completeness of biliary obstruction.

TABLE 32

Blood Plasma Cholesterol—Acute Liver Injury by Chloroform

Dog 32-143.

Date	Weight	Food consumed	Jaundice plasma	Total cholesterol	Esters, cholesterol	Ester ratio
	kg.	*per cent*		*mg. per cent*	*mg. per cent*	*per cent*
Dec. 15	21.0	100	0	96	64	67
Dec. 16		100	0	106	67	62
Dec. 17		100	0	99	62	64
Dec. 18	20.5	100	0	104	67	64
Dec. 19			0	102	65	64
1 hr. $CHCL_3$ anesthesia						
Dec. 20	21.0	100	+++	111	65	59
Dec. 21		100	+++	111	58	55
Dec. 22		100	++	130	78	60
Dec. 23	21.0	100	+	121	76	63
Dec. 24		100	+	93	44	47
Dec. 25		100	+	102	58	57
Dec. 26	20.0	100	Trace	119	62	52
Dec. 27		100	Trace	102	54	53

The effect on blood plasma cholesterol resulting from bile duct obstruction is shown in Table 33, Dog 32-326. The first four lines give average figures for the periods indicated by the dates.

After a control period of a week, the common bile duct was ligated and cut under ether anesthesia. Jaundice developed promptly and the dog began to leave food with resulting slight decrease in weight. Blood plasma cholesterol levels were followed for 27 days. During this period there was no significant change in the ratio of esterified to total cholesterol. The amount of both total and esterified cholesterol, however, did increase definitely above the control levels. Chloroform by mouth was started and immediately there was a rapid decrease in the total blood plasma cholesterol with even more marked drop in the esterified cholesterol.

Consequently the ester ratio falls below normal and remains low. On the last 2 days of life there were no cholesterol esters demonstrable in the blood. 5 days before death when the ester ratio was at 14 per cent the fibrinogen was 109 mg. per cent, indicating very definite liver injury.

Autopsy revealed generalized jaundice with complete obstruction of the common bile duct. The gall bladder and biliary ducts were dilated and filled with thick

TABLE 33

Bile Duct Obstruction with Superimposed Chloroform Injury

Dog 32-326.

Days	Weight	Food consumed	Icterus index	$CHCL_3$ by mouth	Total cholesterol	Esters, cholesterol	Ester ratio
	kg.	*per cent*		*cc.*	*mg. per cent*	*mg. per cent*	*per cent*
Sept. 12–Sept. 18	8.4	100	0	0	172	76	45
Common bile duct ligated and cut							
Sept. 19–Sept. 25	8.6	50	20	0	211	99	49
Sept. 26–Oct. 2	7.5	50	20	0	187	88	46
Oct. 3–Oct. 12	7.3	50	23	0	206	103	51
Oct. 13	7.2	50	23	0	255	127	50
Oct. 14		20		0	335	121	36
Oct. 15		50	21	0	338	125	37
Oct. 16	7.1	50		0	301	165	55
Oct. 17		47	24	4	232	151	65
Oct. 18		30		4	221	73	33
Oct. 19		20	26	4	83	27	33
Oct. 20	7.2	32		0	68	27	39
Oct. 21		20	27	4	53	19	36
Oct. 22		40		0	36	12	34
Oct. 23		6	30	4*	87	12	14
Oct. 24	6.6	0		4	54	14	26
Oct. 25		0	31	8	40	13	33
Oct. 26		0		8	45	12	26
Oct. 27	6.1	0	46	8	45	0	0
Oct. 28				8	46	0	0

* Fibrinogen 109 mg. per cent on this date.

dark green bile. The liver was bile stained and very fatty. No gross evidence of infection was seen. There was a duodenal ulcer. The other organs appeared normal. Histologically the liver showed central fatty degeneration with normal appearing liver cells in the portal regions. Bile canaliculi were distended with brown colloid material. Phagocytic cells in the liver sinusoids contain brown pigment granules.

Table 34, Dog 32-341, shows the results of long continued biliary obstruction with subsequent superimposed chloroform liver injury. Again average figures are given for several periods as indicated by the dates.

This dog was studied for 41 days after obstruction before chloroform was given. During the simple obstructive period there was definite increase in the total and

TABLE 34

Bile Duct Obstruction with Superimposed Chloroform Injury

Dog 32-341.

Date	Weight	Food consumed	Icterus index	$CHCl_3$ by mouth	Fibrinogen	Total cholesterol	Esters, cholesterol	Ester ratio
	kg.	*per cent*		*cc.*	*mg.*	*mg. per cent*	*mg. per cent*	*per cent*
Sept. 12–Sept. 18	13.3	100	0			270	143	52
Common bile duct ligated and cut								
Sept. 19–Sept. 25	15.9	80	10			289	144	50
Sept. 26–Oct. 2	13.8	75	30			388	204	56
Oct. 3–Oct. 9	13.6	70	36			361	180	49
Oct. 10–Oct. 16	12.6	50	42			279	137	49
Oct. 17–Oct. 23	12.4	50	50			381	185	48
Oct. 24–Oct. 30	12.4	100	30			350	193	52
Oct. 31–Nov. 6	12.3	20	52	6		342	170	50
Nov. 7–Nov. 13	11.6	20	54	8	138	111	28	25
Nov. 14	11.0	16	54	10	167	108	23	21
Nov. 15	10.9	18		10		152	27	18
Nov. 16		16	56	20	195	140	25	18
Nov. 17		0				121	25	21
Nov. 18	10.8	10	51		204	110	24	22
Nov. 19		20				164	33	20
Nov. 20	10.5	0	45		306	132	26	20
Nov. 21		0				173	40	23

esterified blood plasma cholesterol above the control levels. The ratio of esters to total cholesterol remains normal. A week after daily administration of chloroform (6 cc.) by mouth the values for both total and esterified cholesterol decrease markedly. The ester ratio drops to 25 per cent of the total as compared with normal of 50 per cent. These lower levels are maintained throughout the rest of the experiment. Food consumption was poor, particularly after chloroform administration was commenced. On Nov. 16 after 10 days of severe liver injury an attempt was made to bring back the dog to normal by administration of intrave-

nous glucose. Apparently the margin of hepatic safety had been passed as the animal died after 4 days of such treatment. Following sugar therapy the fibrinogen levels rose to normal while there was no change in the cholesterol level of the plasma. Before glucose in saline was given on Nov. 16, the dog's red cell hematocrit was at 40 per cent; following glucose this dropped to 20 per cent at which level it remained until death, which occurred despite transfusion. In face of this obviously great dilution of the total plasma volume, there was no drop in the total cholesterol.

TABLE 35

Chronic Bile Duct Obstruction with Superimposed Cholangitis

Dog 31-203.

Date	Weight	Food consumed	Icterus index	Total cholesterol	Esters, cholesterol	Ester ratio
	kg.	*per cent*		*mg. per cent*	*mg. per cent*	*per cent*
July 14	15.7	100	30	315	177	56
July 15		100	30	291	119	41
July 16		100	32	376	184	49
July 17	15.6	100	28	327	164	50
July 18		100	30	322	183	57
Interval of 52 days. Animal now progressively ill						
Sept. 6	15.9	100	28	95	33	35
Sept. 7		100	25	63	28	44
Sept. 8		100	28	81	27	33
Sept. 9		0	35	83	20	24
Sept. 10		100		106	28	26
Sept. 11		100	55	107	24	22
Sept. 12		0	40	57	13	23
Sept. 13		0	45	52	14	26
Sept. 14		0	40	74	13	17
Sept. 15	14.3	0	48	71	13	16

Autopsy showed generalized jaundice, completely obstructed dilated bile ducts filled with dark green bile. The liver was fatty and histologically showed generalized fatty degeneration with scattered liver cells undergoing hyaline necrosis, with resulting polymorphonuclear leucocytic infiltration. Two duodenal ulcers were present. The other organs appeared normal.

Two other experiments of the same type on Dogs 32-270 and 32-380 gave similar results.

Table 35, Dog 31-203, presents some interesting data obtained from study of a chronically obstructed bile fistula dog which had been used

for 18 months on bile salt study and had been totally deprived of bile with the exception of an occasional short period. 12 days after total obstruction occurred, a study of plasma cholesterol was begun.

The table shows a high level for both total cholesterol and esters but a normal ester ratio. For 52 days the dog ate all of its food and continued active, but at the end of this period began to leave food, became inactive and appeared to be going down hill. Cholesterol studies at this time showed a very marked change. Totals of cholesterol are low and the ratio of esters to the total is much below the normal with progressive drop until the level of 16 per cent is reached.

During these last 10 days the dog had three large gastro-intestinal hemorrhages. The red cell hematocrit dropped from 34 to 10 per cent. The animal was very weak and would not eat. Transfusions did not raise the hemoglobin. On the 10th day a large fresh blood clot was vomited. The dog was killed under gas anesthesia, and autopsy performed immediately. The essential findings were generalized jaundice, multiple infected infarcts of varying sizes in the spleen and kidneys. Throughout the gastro-intestinal tract were many fresh and old blood clots. In the duodenum just beyond the pylorus there were two sharply and deeply punched-out ulcers with a central point from which the hemorrhages were occurring.

The liver was studded with abscesses, measuring from 1 cm. to 4 cm. in diameter. The intra-hepatic ducts were distended with pus as were the extra-hepatic and common bile ducts. The ducts had thick walls. The fistula cannula was in place but plugged with inspissated bile. No communication was found between the bile duct and the duodenum. Histologically, the liver showed subacute cholangitis with abscess formation.

Another bile fistula dog (31-331) became totally obstructed and was kept for 7 months on a diet of white bread, klim and water with no bile in the intestinal tract. This animal showed high normal blood plasma cholesterol values with the normal ratio of esters to total cholesterol. These bile fistula animals also maintain a normal cholesterol ester ratio in their blood plasma all through the period in which the fistula is draining and this despite total absence of bile from the intestine.

DISCUSSION

From a consideration of these results it is evident that hypocholesteremia with dissociation of the ratio of esterified to total cholesterol is not due simply to parenchymatous liver injury. In marked acute chloroform liver injury normal values for cholesterol are found whereas

hypocholesteremia occurs in chronic chloroform injury or in injury acutely produced by this drug or infection in dogs with long continued biliary obstruction. One can safely state that the above mentioned changes in blood plasma cholesterol are *related* to chronic severe injury of the liver. This suggests the possibility that these alterations in the metabolism of plasma cholesterol are a secondary manifestation of a chronic derangement of hepatic function.

In all the obstructed dogs we have found a moderate increase in the free and combined cholesterol, but the icterus index has increased from zero to 50 meanwhile. Therefore the increase in cholesterol does not parallel the degree of jaundice as other investigators have maintained. In Paper II it has been shown that in dog's bile there are eliminated about 20 mg. of free cholesterol daily. If, as has been claimed, hypercholesteremia is a result of biliary obstruction alone, then the free cholesterol in the blood should increase much more than the esterified cholesterol, and so dissociation of the ratio of esterified to total cholesterol would be expected. In our dogs a normal ester ratio was maintained during long periods of total obstruction.

The hypercholesteremia observed in biliary obstruction may not necessarily be due to changes within the liver itself. In diabetes and nephrosis high blood cholesterol values are found but this is not attributed directly to changes in the epithelium of the pancreas and kidney.

Quantitative study of blood cholesterol and cholesterol esters has been proposed as a simple test to differentiate between obstructive lesions and parenchymatous lesions of liver. In simple obstruction, the total cholesterol may be elevated with the esters rising in proportion or increasing relatively whereas in parenchymatous injury the total cholesterol will decrease and the combined cholesterol drop even to the point of disappearance in severe injuries. This is true in a great many instances but one must bear in mind the results in acute chloroform poisoning where it has been shown that acute injury does not necessarily change the blood cholesterol.

It should be stressed that in a dog with chronic biliary obstruction with total blood cholesterol values of twice normal (Table 33) this hypercholesteremia may be promptly reduced below normal by chloroform poisoning. Therefore while biliary obstruction may cause high

values for blood cholesterol, the combination of liver injury (chloroform) or biliary infection (Table 35) will cause a prompt fall to subnormal levels. This indicates again the futility of the diagnostic index that hypercholesteremia means bile duct obstruction and hypocholesteremia with dissociation of the ratio of esterified to total cholesterol means liver parenchyma injury.

It is important to emphasize the significance of the ratio of esterified to total cholesterol as a criterion of impairment of liver function over any change that may occur in the total plasma cholesterol. The normal maximum variation which may occur in the total plasma cholesterol is very wide, but the ratio of esterified to total plasma cholesterol is more constant (from 40–70 per cent). The constancy with which this ratio is maintained in dietary extremes and disturbed liver function indicates a physiological process capable of great compensatory effort. When values for the ratio of cholesterol esters of the plasma fall below the "low normal" it is an indication of impairment of the functional capacity of the liver.

It is obvious that the cholesterol analysis like other tests for liver function and liver injury has its limitations and alone will not write the diagnosis for the clinician. The test brings evidence which has weight but must be considered together with all other available data to give a better understanding of liver function and disease.

SUMMARY

Hypocholesteremia with dissociation of the normal ratio of esterified to total cholesterol is related to chronic liver injury caused by chloroform.

Hypercholesteremia may develop after prolonged biliary obstruction.

The hypercholesteremia of chronic biliary obstruction may be promptly reduced below normal by chloroform poisoning or bile duct infection.

Acute injury of liver due to chloroform anesthesia may cause no change in blood plasma cholesterol.

Absence of bile in the intestine with faulty fat absorption does not cause the development of hypocholesteremia with dissociation of the ester ratio.

Poor food consumption or short periods of fasting may cause no change in blood plasma cholesterol.

Liver cells injured by chloroform may subsequently become resistant to chloroform.

After prolonged biliary obstruction, the liver is apparently more sensitive to small doses of chloroform by mouth.

Analysis of blood plasma cholesterol may have a clinical application in differentiation between simple obstructive and parenchymatous lesions of the liver.

BIBLIOGRAPHY

1. Bloor, W. R., *J. Biol. Chem.*, 1916, **24,** 227.
2. Bloor, W. R., personal communication to the authors.
3. Bloor, W. R., and Knudson, A., *J. Biol. Chem.*, 1916, **27,** 107.
4. Bruger, M., and Somach, I., *J. Biol. Chem.*, 1932, **97,** 23.
5. Cutten, C., Emerson, E. E., and Woodruff, W., *Arch. Int. Med.*, 1928, **41,** 428.
6. Epstein, E. Z., *Arch. Int. Med.*, 1932, **50,** 203.
7. Feigl, J., *Biochem. Z.*, 1918, **86,** 1.
8. Gardner, J. A., and Gainsborough, H., *Quart. J. Med.*, 1930, **23,** 465.
9. Gardner, J. A., and Gainsborough, H., *Biochem. J.*, 1928, **22,** 1048.
10. Jones, T. B., and Smith, H. P., *Am. J. Physiol.*, 1930, **94,** 144.
11. MacNider, W. deB., *Proc. Soc. Exp. Biol. and Med.*, 1932, **30,** 328.
12. Mjassnikow, A. L., *Klin. Woch.*, 1932, **11,** 1910.
13. Rous, P., and McMaster, P. D., *J. Exp. Med.*, 1923, **37,** 11.
14. Thannhauser, S. J., *Deutsch. Arch. klin. Med.*, 1922, **141,** 290.
15. Thannhauser, S. J., and Schaber, H., *Klin. Woch.*, 1926, **5,** 252.