Lipoproteins, Cholesterol and Phospholipids in Sera of Rabbits Following Ligation of the Common Bile Duct or Injection of Carbon Tetrachloride

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In pediatric practice it was not infrequent to meet with a difficulty in etiological diagnosis for neonatal jaundice. For example, both neonatal hepatitis and atresia of the bile duct would present similar clinical and laboratory findings of "obstructive jaundice", and while an early surgical treatment is recommended for the cases with atresia of the bile duct, the therapy for those of neonatal hepatitis will be quite different.

In the present investigation it was tried to investigate whether or not any difference of lipoprotein patterns, cholesterol and phospholipids would be found between rabbits with ligation of the common bile duct, which may be considered as an extreme case of biliary obstruction on one side and those with severe damage on hepatic parenchyma produced by carbon tetrachloride injection on the other.

MATERIALS AND METHODS

The methods for determination of cholesterol and phospholipids in serum were Zak's¹⁾ and Furukawa's²⁾ methods respectively. Paperelectrophoretic analysis of serum lipoproteins was done in the same way as described in a preceding paper of mine.³⁾ Rabbits, weighing 2,000-2,500 g, were used in this investigation.

Method for animal experimentation: — Carbon tetrachloride, 0.5 cc per kg of body weight, was injected into 4 rabbits subcutaneously for successive two days. The first blood specimen was obtained just prior to the first injection of carbon tetrachloride, and on the next day the second blood specimen was taken, immediately after which the second injection was done, and the third blood specimen was obtained on the third day after the second injection, i.e. on the fourth day of the first injection.

Ligation of the common bile duct was performed on 3 rabbits, and blood

examination was done just prior to the operation for the first time, and on one and three days after the ligation.

RESULTS AND DISCUSSION

The results obtained were shown in Tables I-V. Changes in cholesterol, phospholipids and lipoprotein pattern following the injection of carbon tetrachloride and the ligation of the common bile duct will be discussed on the basis of average values for each experimental group.

TABLE I. Influence of Injection of Carbon Tetrachloride upon Cholesterol in Sera of Rabbits

No. of blood specimens	I Before the injection		II One day after the first injection		III 3 days after the second injection	
No. of rabbits	Cholesterol (mg/100 ml)		Cholesterol (mg/100 ml)		Cholesterol (mg/100 ml)	
	Total	Free (%)	Total	Free (%)	Total	Free (%)
3	168	23(13)	58	48(82)	83	45(54)
4	75	25(33)	45	40(88)	103	93(90)
6	58	25(43)	40	10(20)	78	53(67)
7	110	52(46)	85	28(33)	178	65(36)
Average	102	31(30)	57	31(55)	110	64(61)

TABLE II. Influence of Injection of Carbon Tetrachloride upon Phospholipids in Sera of Rabbits

No. of blood specimens	I Before the injection	II One day after the first injection	III 3 days after the second injection
No. of rabbits	Phospholipids (mg/100 ml)	Phospholipids (mg/100 ml)	Phospholipids (mg/100 ml)
3	105	105	107
4	115	125	167
6	65	32	177
7	86	43	72
Average	92	76	130

Cholesterol (cf. Fig. 1): — Total cholesterol did not show remarkable changes within 3 days after the bile duct ligation, while, in the case of carbon tetrachloride injection, it showed a marked decrease on the next day and rose slightly above the preinjection level. The ratio of free to total cholesterol increased in both experimental groups and more conspicuously in the case of the bile duct ligation.

TABLE III. Influence of Injection of Carbon Tetrachloride upon Lipoproteins in Sera of Rabbits

No. of blood specimens	I Before the injection					II One day after the first injection					III 3 days after the second injection				
No. of rabbits	Lipoproteins (%)			α/β	α/γ	Lipoproteins (%)			α/β	α/γ	Lipoproteins (%)			α/β	α/γ
	α	β	γ			α	β	γ			α	β	γ		
3	33.9	24.1	42.0	1.41	0.80	12.8	31.7	55.5	0.40	0.23	25.3	45.8	28.9	0.55	0.87
4	37.4	44.6	18.0	0.84	2.07	12.2	34.1	53.7	0.36	0.20	14.4	54.2	31.4	0.27	0.48
6	30.3	41.9	27.8	0.72	1.35	17.4	37.6	45.0	0.46	0.38	4.4	66.7	28.9	0.07	0.15
7	21.6	39.9	38.4	0.54	0.56	25.4	37.5	37.1	0.68	0.68	9.7	61.9	28.4	0.16	0.34
Average	30.8	37.6	31.5	0.87	1.19	16.9	35.2	47.8	0.47	0.37	13.4	57.1	29.4	0.26	0.46

TABLE IV. Influence of Ligation of the Common Bile Duct upon Cholesterol and Phospholipids in Sera of Rabbits

Conditions at the time of blood sampling	Before the ligation			After ligation of the common bile duct					
				One day			Three days		
No. of rabbits	Cholesterol (mg/100 ml)		Phospholipids (mg/100 ml)	Cholesterol (mg/100 ml)		Phospholipids (mg/100 ml)	Cholesterol (mg/100 ml)		Phospholipids (mg/100 ml)
	Total	Free(%)		Total	Free(%)		Total	Free(%)	
8	120	50(41)	43.4	103	68(66)	92.5	89	78(87)	52.1
9	55	25(45)	64.8	90	88(97)	83.3	125	125(100)	92.5
10	80	35(43)	64.8	50	50(100)	55.5	45	45(100)	111.1
Average	85	36(43)	57.6	81	68(87)	77.1	86.3	82(96)	85.2

TABLE V. Influence of Ligation of the Common Bile Duct upon Lipoproteins in Sera of Rabbits

Conditions at the time of blood sampling	Before ligation of the common bile duct					Three days after ligation of the common bile duct				
No. of rabbits	Lipoproteins (%)			α/β	α/γ	Lipoproteins (%)			α/β	α/γ
	α	β	γ			α	β	γ		
8	17.4	49.8	32.8	0.35	0.53	19.4	62.0	18.5	0.31	1.05
9	24.7	46.5	30.8	0.53	0.80	14.1	72.5	13.4	0.19	1.05
10	22.1	48.6	29.3	0.45	0.75	16.4	54.0	29.6	0.30	0.55
Average	21.3	48.3	30.9	0.44	0.69	16.6	62.8	20.5	0.26	0.88

Fig. 1. Serum cholesterol following the bile duct ligation and following injection of carbon tetrachloride in rabbits.

○—○ Total cholesterol } in case of ligation of the common bile duct
 △—△ Free cholesterol }
 ●—● Total cholesterol } in case of injection of carbon tetrachloride
 ▲—▲ Free cholesterol }

Phospholipids (cf. Fig. 2): — A steady increase in phospholipids in serum was found in case of the bile duct ligation, while, in case of carbon tetrachloride injection, a marked increase in phospholipids was attained on the 4th day after the first injection, accompanied by a marked decrease on the next day of the first injection.

Lipoprotein pattern (cf. Fig. 3): — A decrease in α -lipoprotein associated with an increase in β -lipoprotein was found in both the experimental groups, but it was worthy of note that the change in γ -lipoprotein differed in each group, that is, a decrease in γ -lipoprotein was observed in case of the bile duct ligation, while no change in this fraction was seen in case of the carbon tetrachloride injection. This relation was more clearly demonstrated in Fig. 3, in which the ratio of α -to γ -lipoprotein (α/γ) did not decrease, but tended to increase in case of the

Fig. 2. Serum phospholipids following the bile duct ligation and following injection of carbon tetrachloride in rabbits.

○—○: in case of the bile duct ligation

●—●: in case of injection of carbon tetrachloride

bile duct ligation on one hand, and the α/γ ratio decreased markedly in case of the carbon tetrachloride injection on the other hand.

In rats with the ligation of bile duct Chanutin and Ludewig⁴⁾ demonstrated an increase in serum free cholesterol with no increase in ester cholesterol and an increase in phospholipids proportional to the increase in free cholesterol. Hawkins and Wright⁵⁾ reported, however, that in dogs the ligation of the common bile duct caused an increase in the absolute amount of cholesterol ester which paralleled to the hypercholesteremia and that cholesterol ester was reduced in these animals only after superimposing added damage by an administration of chloroform.

In 1950 Albrink *et al.*,⁶⁾ basing upon clinical investigations of hepatitis and obstructive jaundice, reported that the ratio of free to total cholesterol rose markedly in both conditions and that the total cholesterol was the lowest where the element of parenchymal damage was the greatest, that is, in severe infectious hepatitis, and further that it was higher where the element of obstruction was the greatest, in prolonged biliary obstruction.

In 1955 Eder *et al.*,⁷⁾ reported the results of clinical investigation into biliary cirrhosis, obstructive jaundice and acute hepatitis, in which a decrease in α -

Fig. 3. The ratios of α/β and of α/γ in sera from rabbits with CCl_4 injection or ligation of the common bile duct.

○—○ : Rabbits with ligation of the common bile duct
 ●---● : Rabbits with the CCl_4 injections

lipoprotein associated with an increase in β -lipoprotein was observed paper electrophoretically in all these conditions. Furthermore they carried out chemical fractionation of plasma protein by using Cohn's method and found an occurrence of abnormal lipoprotein of which a high level was found in obstructive jaundice but not in hepatitis.

A finding to be noticed in my experimentation is the change in γ -lipoprotein, that is, γ -lipoprotein was decreased markedly following the bile duct ligation, but did not decrease following the carbon tetrachloride injection. Most part of lipids in γ -lipoprotein consisted of triglyceride and chylomylon⁸⁾ which were derived from dietary fat. It was therefore easily acceptable that an impaired absorption of dietary fat from intestine due to block of the bile flow by the bile duct ligation brought about a decrease in γ -lipoprotein in serum.

SUMMARY AND CONCLUSIONS

An increase in total cholesterol, the ratio of free to total cholesterol and

phospholipids and a decrease in the ratio of α -to β -lipoprotein in serum were observed similarly in both experimental conditions of rabbits — the bile duct ligation and the injection of carbon tetrachloride. But a decrease in γ -lipoprotein was observed in case of the bile duct ligation only, but not in case of the injection of carbon tetrachloride.

References

- 1) Zak, B. *et al.*, Am. J. Med. Technol., 1957, **23**, 283.
 - 2) Furukawa, M. *et al.*, Seikagaku, (Jap.), 1952, **24**, 76.
 - 3) Chida, N., in press in this Journal.
 - 4) Chanutin, A. & Ludewig, S., J. Biol. Chem., 1936, **115**, 1.
 - 5) Hawkins, W.B. & Wright, A., J. Exp. Med., 1934, **59**, 427.
 - 6) Albrink, M. J. *et al.*, J. Clin. Inv., 1950, **29**, 781.
 - 7) Eder, H.A. *et al.*, *ibid.*, 1955, **34**, 1147.
 - 8) Knorr, D. & Wehrheim, I.E., Zeitschr. f. K.H.K., 1961, **85**, 308.
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