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Effect of Taurine on Metabolic Function of Isolated, Perfused Rat Liver[1]

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Effect of administered taurine on metabolic function of the liver was investigated by the isolated, perfused livers from normal and CCl_4-poisoned rats.

With the infusion of 1 mM taurine, both the normal control and the CCl_4-damaged livers showed the significant increases in biliary excretion of both total cholesterol and congo red during the perfusion. On the other hand, the stimulation of glycogen synthesis and the suppression of lactic acid production were observed in the normal livers, while the inhibition of the release of triglyceride into the perfusate and the promotion of its accumulation in the tissue were seen in the damaged livers.

The most well-known function of taurine is to conjugate with bile acids in the liver of many mammals.[3] Administered taurine is greatly concentrated in the liver and conjugates with bile acids, as does endogenous taurine.[4] O'Maille, *et al.* reported[5] that acute taurine depletion produced in the livers of bile duct-cannulated dogs by constant infusion of cholic acid, could be reversed following injection of taurine. Since bile acids are the main metabolic end products of cholesterol in the liver, administered taurine may influence on liver functions relating to cholesterol metabolism through the conjugation mechanism with bile acids.

The preceding work of this laboratory demonstrated that a long term-feeding of taurine causes to normalize the free fatty acid composition of the liver lipid of hereditary hyperglycemic obese mice.[6]

Isolated liver perfusion is a useful technique for investigation of liver metabolic functions, without any influence of other organs.

The present paper deals with the effect of taurine on changes of sugar and lipid components occurring during perfusion in the blood, liver tissue and bile of isolated livers from adult normal and carbontetrachloride-treated rats and in addition on dye-clearance activity of these livers.

Material and Method

Taurine——Taurine used here was a synthetic product of J. P. grade, which was dissolved in distilled water as 125 mg (1.0 mM) per ml.

Animals——Male Wistar rats weighing approximately 400 g were used as blood and liver donors. They were fed a commercial diet (CLEA, CE-2) and water *ad libitum*.

Perfusion Medium——The perfusion medium used in the experiment was similar to that of Brauer, *et al.*[7] Rat whole blood was obtained from the abdominal aorta, defibrinated, filtered and stored over-

1) This work was partly presented at the 90th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, July, 1970.
2) Location: *Takata-3-chome, Toshimaku, Tokyo.*
3) G. Haslewood, "Bile Salts," ed. by R. Peters and F. Young, Methuen & Co., Ltd., London, 1967, pp. 82—106.
4) *a*) J. Sjövall, *Proc. Soc. Exp. Biol. Med.*, **100**, 676 (1959); *b*) O. Portman and G. Mann, *J. Biol. Chem.*, **213**, 733 (1955); *c*) A. Minato, S. Hirose, T. Ogiso, K. Uda, Y. Takigawa and E. Fujihira, *Chem. Pharm. Bull.* (Tokyo), **17**, 1498 (1969).
5) E. O'Maille, T. Richards and A. Short, *J. Physiol.* (London), **180**, 67 (1965).
6) E. Fujihira, H. Takahashi and M. Nakazawa, *Chem. Pharm. Bull.* (Tokyo), **18**, 1636 (1970).
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night at 4°. To the defibrinated blood, potassium penicillin G and dihydrostreptomycin sulfate were added as antiseptic in 0.03% and 0.02% respectively. The stock blood was diluted with two volumes of the Locke's solution containing bovine serum albumin (Armour, Fraction V) in 5% and the pH was adjusted to 7.4 with phosphate buffer. The volume of the diluted blood for perfusion was fixed to 128 ml.

Liver Perfusion——The perfusion system employed here is shown diagrammatically in Fig. 1. All glassware were coated inside with silicone. Siliconized tubes were used as connector. Perfusion pump used was of a piston-type (Sanyo Rikagaku Kikai, T-63-K-100). The small upper reservior flask was employed to maintain the flowing to the liver at a constant pressure of 120 mm by overflow bypass. The flow rate was arbitrarily checked by an electromagnetic flowmeter (Nihon Kohden, MF-2). The perfusion medium was equilibrated with 95% O_2–5% CO_2 and maintained at a constant temperature of 38±1° during the perfusion. The liver for perfusion was taken out and set to the circuit as follows. The abdomen of the liver donor was opened after pentobarbital anesthesia. The common bile duct was cannulated at a distal portion of the liver. The portal vein was ligated and rapidly cannulated after the injection of heparin (300 unit). The thorax was opened and the inferior vena cava cut off from the heart. The liver with the attached diaphragm was rapidly dissected out, placed diaphragmatic side down in a round nylon net with a 5 mm hole in the bottom through which the vena cava was passed, and connected to the perfusion circuit. The time from ligating of the portal vein until the start of perfusion was 4—5 min. The livers showing the outflow rate less than 30 ml per min were discarded from the experiment. The carbontetrachloride(CCl_4)-damaged livers were obtained from the rats which had subcutaneously given 1.6 g per kg of CCl_4 24 hr before the experiment.

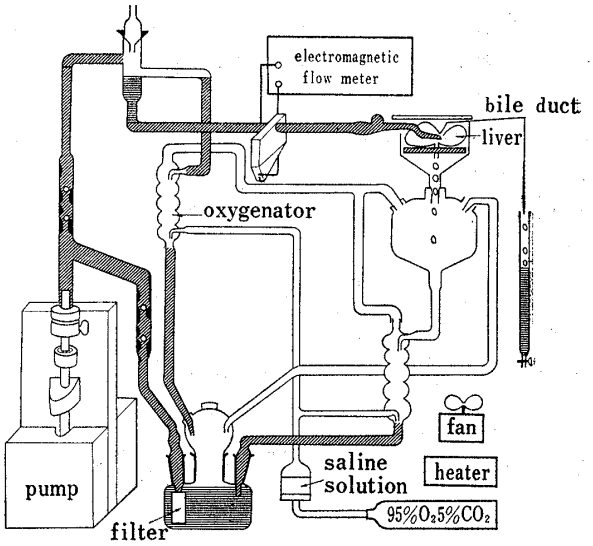

Fig. 1. The Perfusion Apparatus

Taurine Infusion and Sampling Time——Sixty minutes after the start of perfusion, 1 ml of the taurine solution was added rapidly to the main reservoir flask and mixed thoroughly with the perfusion medium. Blood specimens (2 ml per each) were removed from the main reservoir flask, 60, 90, 120 and 150 min after the start of perfusion. The 60 min-blood was used as zero-time sample. For the same intervals, bile was collected in a small measuring tube with a stop cock at the bottom. Liver specimens (approximately 100 mg per each) were taken out 30 min after the start of perfusion, as zero-time, and thereafter for the 1 hr intervals. In the congo red clearance test, taurine (1 mM) was infused 30 min after the start of perfusion, together with 2 mg of congo red (Merck). Perfusing blood was removed 60 and 120 min after the infusion and bile was collected for the same interval.

Chemical Analysis——Blood samples were centrifuged and the plasma were separated for the following assays. Glucose was routinely determined by the glucose oxidase method as modified for use with a Pye-Unicam autoanalyser (AC-60).[8] Lactic acid assay was carried out according to the method of Barker, *et al.*[9] Free fatty acid levels were determined by the method of Itaya, *et al.*[10] Total cholesterol assay was carried out according to the method of Rosenthal.[11] Total triglyceride was determined using the Wako Kit for Triglyceride Test based on the method of Fletcher.[12] The liver tissue taken out was cut into two pieces. One piece, immediately after weighing, was immersed into 0.5 ml of a hot 30% KOH solution and digested by heating. The resulting glucose was determined enzymatically using the autoanalyser. Liver lipids were extracted from another piece with a mixture of MeOH and chloroform (1: 2) and assayed for total cholesterol and triglyceride. Cholic acid was determined by a modified Pettenkofer's method.[13] Biliary total cholesterol was assayed on the lipid extracts from bile. The amounts of congo red retained in blood or excreted in bile were determined at the extinction of 500 mμ.[14]

8) Pye-Unicam Automatic Analysis Method No. 3: Determination of Glucose in Blood Serum.
9) S. Barker and W. Summerson, *J. Biol. Chem.*, **138**, 535 (1941).
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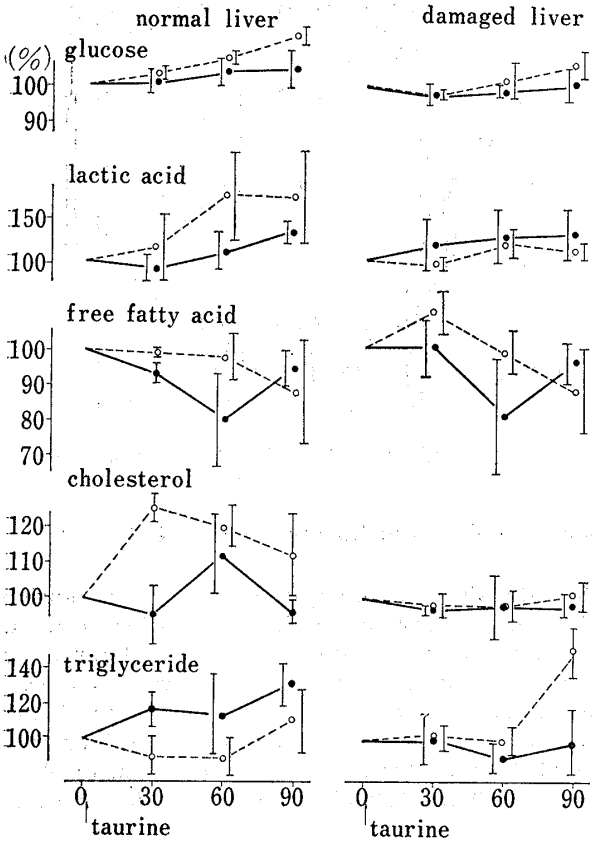
Result

Table I shows the zero-time value of each component in perfusate, bile or liver tissue. As compared with the normal controls, the CCl_4-damaged livers showed significantly low levels of perfusate cholesterol, biliary cholesterol and liver glycogen, and in contrast a considerably high content of liver triglyceride. On the other hand, no significant differences in other components were found between the normal and the CCl_4-damaged livers, compared to zero-time values.

Figure 2 shows the effect of taurine infusion on the changes of perfusate components during the consecutive 90 min-perfusion after zero-time sampling. With the normal controls, perfusate glucose and lactic acid levels slightly increased from the starting values, while the free fatty acid level progressively decreased during the perfusion. After taurine infusion, these changes overall diminished and the starting levels lasted through the perfusion period. With the CCl_4-damaged livers, there were only minimum increases of glucose and lactic acid and a slight increase followed by a gradual decrease of free fatty acid during the perfusion. These were not significantly affected with the infusion of taurine.



time (min) after zero-time sampling with taurine (-•-); without taurine (-o-)

Fig. 2. Changes in Level of Perfusate Components during Perfusion

Arrow indicates the time of taurine infusion and bar represents standard error of the mean; six determinations each for normal liver and four determinations each for damaged liever. The zero-time levels are fixed arbitrarily at 100%.

As for the lipid components, average 26% increase of total cholesterol in the first 30 min and gradual decrease in the next 60 min were observed during the perfusion of the normal livers. Following taurine infusion, this initial rise disappeared and a slight increase occurred only in the 60 min. In the contrast, no increase of cholesterol was observed during the perfusion of the CCl_4-damaged livers and the starting level lasted throughout the experimental period, with or without taurine. Triglyceride level increased only in the last 30 min of perfusion period to a lesser extent with the normal controls, and to a greater extent with the damaged livers. Following taurine infusion, perfusate triglyceride tended to be slightly increased in the former, while that remained unchanged in the latter.

Figure 3 shows the changes in content of glycogen, total cholesterol and triglyceride of liver after zero-time sampling.

With the normal livers, the glycogen content did not alter in the first 60 min and then in the next 60 min decreased to less than 50% of the starting level. In the earlier phase after taurine infusion, a significant rise followed by a rapid fall was observed in the glycogen. In the case of the CCl_4-damaged livers, on the other hand, the content of glycogen remained almost unchanged during perfusion and this was not affected with the infusion of taurine.

The liver contents of total cholesterol and triglyceride considerably varied from sample to sample in both cases of the normal and the CCl_4-damaged livers with or without taurine.

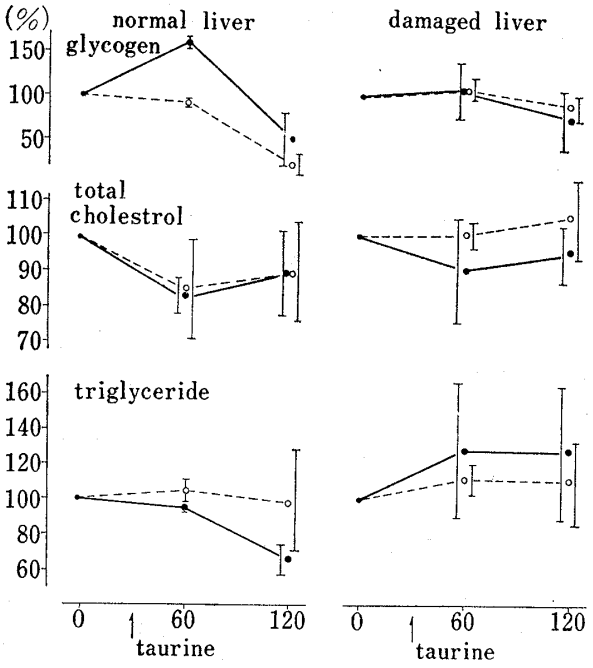
It was only noted that the cholesterol content of the damaged livers tended to decrease after taurine infusion, while the triglyceride content progressively increased with large variations.

Both the biliary excretion of total cholesterol and cholic acid, and the bile flow rate, are shown in Fig. 4. In either case of the normal or the CCl_4-damaged livers, the biliary

Table I. Starting Levels of the Components measured in Perfusate, Liver Tissue and Bile

Component	(Unit)	Normal liver[a)]	CCl_4-damaged liver[b)]
Perfusate			
glucose	(mg/dl)	263 ±19	199 ±16
lactic acid	(mg/dl)	14.4 ± 3.0	19.2 ± 3.7
free fatty acid	(μeq/dl)	20.9 ± 2.0	17.7 ± 1.1
cholesterol	(mg/dl)	95 ±12	50 ± 3
triglyceride	(mg/dl)	29 ± 3	23 ± 1
Liver			
glycogen	(mg/g)	22.7 ± 3.7	14.0 ± 3.1
cholesterol	(mg/g)	4.78± 0.41	5.18± 0.38
triglyceride	(mg/g)	8.80± 1.55	19.30± 3.10
Bile			
volume	(ml/30 min)	0.49± 0.03	0.56± 0.03
cholesterol	(μg/30 min.)	20 ± 3	6 ± 1
cholate	(mg/30 min.)	0.43± 0.08	0.50± 0.03

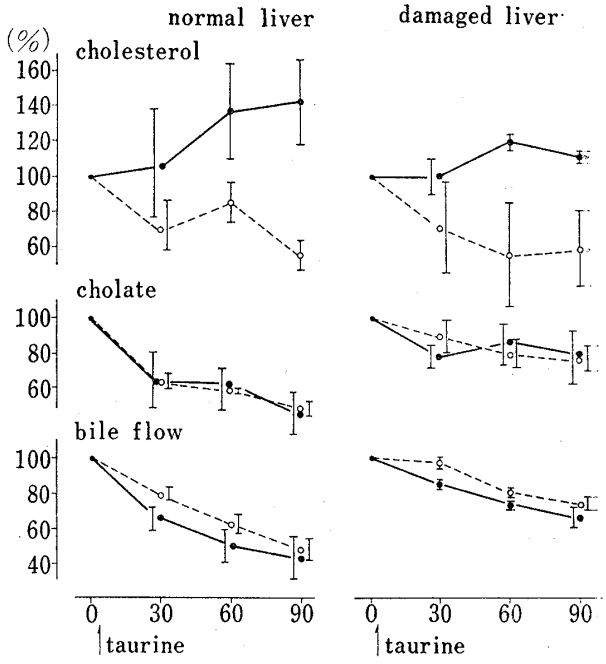
a) average of 12 determinations ±S.E.
b) average of 8 determinations ±S.E.



time (min) after zero-time sampling with taurine (-•-); without taurine (-o-)

Fig. 3. Changes in Content of Liver Components during Perfusion

Arrow indicates the time of taurine infusion and bar represents standard error of the mean; six determinations each for normal liver and four determinations each for damaged liver. The zero-time contents are fixed arbitrarily at 100%.



time (min) after zero-time sampling with taurine (-•-); without taurine (-o-)

Fig. 4. Changes in Biliary Excretion of Total Cholesterol and Cholic Acid Conjugates and in Bile Flow Rate during Perfusion

Arrow indicates the time of taurine infusion and bar represents standard error of the mean; six determinations each for normal liver and four determinations each for damaged liver. The zero-time values are fixed arbitrarily at 100%.

excretion of cholesterol and cholic acid progressively decreased during the perfusion, paralleling with the decrease of bile outflow. The infusion of taurine produced a marked promotion of the biliary excretion of total cholesterol in the normal controls, while that resulted in a sustained but rather slightly increased excretion of the biliary cholesterol in the CCl_4-damaged livers. However, the biliary excretion pattern of cholic acid was not modified with taurine infusion and the bile flow rate slightly decreased after the infusion in either case.

Table II. Effect of Taurine on Congo Red Activity of Isolated Perfused Liver from Normal and CCl_4-treated Rats

	Perfusate (dye-retention %) ±S.E.[a]		Bile (dye-excretion %) ±S.E.[b]	
	60 min	120 min	60 min	120 min
Normal				
control	28.4±2.3	17.6±5.6	8.1±1.9	26.7±2.5
taurine	27.3±4.1	17.1±6.1	8.2±1.1	38.0±3.5
Damaged				
control	27.7±3.5	23.1±3.3	5.8±0.7	20.3±3.3
taurine	29.6±3.5	19.8±6.1	8.1±1.1	27.3±1.7

a) amount retained in perfusate per dye added (2 mg)
b) amount excreted in bile per dye added
* average of 4 determinations

The results of the congo red-clearance test are shown in Table II. The clearance rate of congo red from perfusing blood was not different between the normal and the CCl_4-damaged livers, while the excretion rate of congo red into bile was relatively faster in the former than in the latter. The simultaneous infusion of taurine with congo red resulted in no changes of the clearance activity during the perfusion but significantly stimulated the biliary excretion of the dye in either case.

Discussion

Bile flow rate is an important criterion to determine whether the function of the isolated, perfused liver is physiologically normal.[7,15] In this respect, the base-line data obtained here are fairly comparable to those described in the literature.[16]

In the present study, it is clearly found that taurine infusion causes significant increases in biliary excretion of total cholesterol and congo red in both cases of the livers from the normal and the CCl_4-treated rats.

Entenmann, *et al.*[17] described that with the isolated, perfused rat liver, the infusion of bile acids resulted in a significant stimulation of the release of phospholipid and free cholesterol into bile.

Oxidation to bile acids is the most important pathway for the elimination of cholesterol in rats.[18] Bile acids are ordinally excreted in the bile as the conjugates with taurine or glycine.[3] Some workers show evidence that administered taurine, but not glycine, significantly

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increases the normal ratio of tauro- to glyco-bile acids (T/G ratio) in the bile of the human and the rat.[19] Brauser, *et al.*[20] demonstrated that the incorporation of radioactivity from ^{14}C-acetate into taurocholic acid in the rat bile was increased 3 to 4 times greater than control after the infusion of 1 mM taurine into a perfusion system of the isolated liver. It is known, however, that the rat bile contains the other two trihydroxycholanoic acids than cholic acid, α- and β-muricholic acids which are derived primarily and secondarily from the alternative metabolic pathway of cholesterol *via* chenodeoxycholic acid.[21] It seems that the tauro-trihydroxycholanoic acids of the rat bile could not be separated each other by such a paper-chromatographic method[22] as used in the experiment of Brauser, *et al.*[20] On the other hand, Pettenkofer's reaction is only positive for cholic acid.[23] The present study shows that the biliary excretion of cholic acid was almost unchanged with the infusion of 1 mM taurine in both cases of the perfusion experiments. However, there is a possibility that taurine might influence on the biliary excretion of the other two trihydroxycholanoic acids. Some investigations on this problem are now in progress.

Congo red clearance activity is essentially due to the reticuloendothelial function of the body, especially of the liver, since the congo red injected is exclusively accumulated in this organ, taken up by Kupffer's cell and excreted into the bile.[24] While, a close relation has been found between the activity of Kupffer's cell and the degradation and excretion of cholesterol in the liver.[25] For these reasons, it seems comprehensible that there were the associated increases in biliary excretion of both cholesterol and congo red after taurine infusion.

Besides, taurine infusion causes the increase of glycogen synthesis and the decrease of lactic acid production in the normal state, while that produces the suppression of the release of triglyceride into perfusing blood and its accumulation in the liver tissue in the CCl_4-damaged state, although the hepatic synthesis of triglyceride in CCl_4-poisoning may considerably vary from phase to phase.[26]

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