Studies on Myoinositol

IV. Effect of Myoinositol on the Cholesterol Metabolism of Rats Suffering from Experimental Fatty Liver

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(Received July 3, 1967)

The administration of a fat free, low protein diet with no B vitamin supplement to albino rats for 3 weeks resulted in marked fatty livers. When these depleted rats suffering from fatty liver were treated with B vitamins for a week, livers were abnormally swelled and their fats and cholesterol were greatly increased. Such a type of fatty liver, however, was partly cured by administering, besides B vitamins, either myoinositol or choline, and almost completely by administering both of them for a week.

A considerable delay was found in the movement of cholesterol from fatty livers to adipose tissues, when compared with that from the livers cured by administration of myoinositol. This may be ascribed to an occurrence of an inhibitory mechanism on cholesterol transport in fatty livers, which is eliminated by the administration of myoinositol.

It is well-known that fatty livers could be produced by dietary regimes such as starvation, insufficient or poor-quality protein, large amounts of carbohydrates, massive doses of saturated fat or cholesterol, and dietary deficiencies or imbalances in vitamin B complex. McHenry and co-workers (1-6) and Handler (7) reported a marked development of fatty liver, when rats, previously stunted by administering a depletion diet, were furnished with B vitamins for a week. The accumulation of fat and cholesterol in the liver was prevented by simultaneous administration of myoinositol.

To clarify such lipotropic action of myoinositol in more detail, however, it may be essential to investigate, from the biochemical and histological point of view, how to develop this type of fatty liver and how to prevent it by the administration of myoinositol. Our attention has been focused on the fact that a large amount of cholesterol is deposited in the fatty liver and that in the animal body, the liver is the principal site for cholesterol biosynthesis (8-11). It is possible to follow the mobilization of cholesterol from the liver by measuring the radioactivity

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of ^{14}C -cholesterol formed from ^{14}C -acetate *in vivo*. The main purpose of the present study is to show some effect of myoinositol administration on the delayed cholesterol mobilization from the liver to adipose tissue in rats suffering from fatty liver. Histological evidence for the lipotropic action of myoinositol is also described.

EXPERIMENTAL

1. Rearing of Animals

Male albino rats of Wistar strain, weighing about 100 g, were used. Animals were housed in individual cages and fed on Handler's depletion diet (7) consisting of 10 % casein, 84 % sucrose, 4 % salts² and 2 % cellulose powder (free from B vitamins, fats and cholesterol), for 3 weeks. The stunted animals were then divided into four groups. The first group was given only B vitamins (thiamine 25 μg , riboflavin 30 μg , calcium pantothenate 100 μg , pyridoxine hydrochloride 20 μg , nicotinamide 50 μg and *p*-aminobenzoic acid 100 μg) (7) by subcutaneous injection daily (group A). Besides B vitamin mixture, the other three groups were given either 30 mg of myoinositol (group B) or 30 mg of choline chloride (group C), or both of them (group D). These treatments were continued for a week.

At each stage of rearing, some rats were sacrificed to prepare the hepatic tissue specimen. Each specimen was stained with Sudan III.

2. Administration of 1- ^{14}C -Acetate

After a week's treatment of stunted animals with B vitamins with or without lipotropic agents, each experimental group (group A-D) was injected intraperitoneally with 15 μCi per rat of 1- ^{14}C -acetate (purchased from Daiichi Pure Chemicals Co.). At certain times after injection (1, 3, 10 and 24 hours), rats were sacrificed by decapitation, and the specific radioactivity of labeled cholesterol in the liver and in adipose tissue was analyzed.

3. Analysis of Liver Lipids

At each stage of rearing, liver total lipids and cholesterols were analyzed as reported previously (12).

4. Analysis of Cholesterol

Total tissue lipids were extracted as reported in a preceding paper (13). After saponification of extracted lipids, free cholesterol was separated on thin layer chromatogram (Kieselgel G nach Stahl) using a mixture of benzene and ether (4:1, v/v) as a developer and eluted with chloroform-methanol (3:1, v/v). An aliquot of the eluate was used for the estimation of cholesterol according to Zlatkis (14). Another aliquot was used for the estimation of radioactivity, by use of a type 03T-004 liquid scintillation spectrometer of Kobe Industrial Co. Specific radioactivity was expressed as cpm/mg cholesterol. Scintillator used was prepared according to Bray (15).

RESULTS

1. Lipotropic Action of Myoinositol and Choline

When rats are stunted by feeding on low protein, high carbohydrate diet with

² The salt mixture consists of 7.5 % $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 9.9 % $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 27.0 % K_2HPO_4 , 15.3 % $\text{Ca}_3(\text{PO}_4)_2$, 36.9 % calcium lactate and 3.4 % ferrous citrate $\cdot 5\text{H}_2\text{O}$.

no vitamins, considerable fats are infiltrated in their livers. As shown in Photograph 1-II, depositions of fats are found in the whole range of livers of stunted animals, in marked contrast to rats fed on normal diets (Photograph 1-I).

To cure these animals suffering from fatty liver, however, administration of only B vitamins was not effective as shown in Photograph 1-III; this resulted in no improvement. Livers swelled abnormally and displayed a milky white color, due probably to the promotion of fat synthesis by a supplement of B vitamins. In fact, considerable increase in amounts of hepatic lipid was found (Table 1) as reported by other workers (1-7).

When stunted rats were given myoinositol simultaneously with B vitamins, however, a significant improvement was found in the liver situation (Photograph 1-IV). In this case, deposition of fat was found mainly at the periphery of the lobule of the liver, whereas deposition of fat around the central vein was scarcely found. A similar result was obtained when stunted rats were given choline chloride instead of myoinositol (Photograph 1-V).

A perfect recovery from fatty liver was found when stunted animals were given both choline and myoinositol. Histological examination showed that there was no significant difference in appearance between the liver of rat administered with both choline and myoinositol (Photograph 1-VI) and that of normal rat (Photograph 1-I). This suggests a synergistic action (16) of choline and myoinositol in the removal of deposited fat from fatty liver.

An analysis of the hepatic total lipids and cholesterol of normal and stunted (suffering from fatty liver) rats are summarized in Table 1. In this table analyses are also reported for animals given B vitamins with or without lipotropic agents. These results were in good agreement with the histological experiments mentioned above.

2. Effect of Lipotropic Agents on the Cholesterol Metabolism of Rat Suffering from Fatty Liver

As indicated in Table 1, liver cholesterol level was fairly elevated when rats suffered from fatty liver. Since no cholesterol was given orally to rats throughout the experimental period, it may be difficult to suppose that the origin of this excess cholesterol is exogeneous. Accordingly, there may be at least two possibilities to explain the high level of hepatic cholesterol: (a) the promotion of cholesterol biosynthesis in fatty liver and (b) extension of the retention time of endogeneous

TABLE 1
Analytical Data on Hepatic Lipids of Various Experimental Groups

Experimental group	Addition	Liver wet weight	Liver total lipids	Liver total cholesterol
		<i>g</i>	<i>g</i>	<i>mg</i>
Normal	—	3.5 ± 0.4	0.19 ± 0.03	—
Depleted	—	3.3 ± 0.5	0.76 ± 0.15	—
A	B vitamins	6.1 ± 0.4	1.11 ± 0.17	25.9 ± 2.7
B	B vitamins + myoinositol	4.4 ± 0.2	0.37 ± 0.08	16.8 ± 1.9
C	B vitamins + choline	4.8 ± 0.6	0.51 ± 0.09	17.9 ± 1.2
D	B vitamins + myoinositol + choline	4.1 ± 0.3	0.25 ± 0.07	15.1 ± 1.6

cholesterol in fatty liver probably due to damage of the hepatic cholesterol transport system.

To test these assumptions, the cholesterol biosynthesis and its movement from the liver were examined in rats suffering from fatty liver and in rats cured by administration of the lipotropic agents, by using ^{14}C -acetate as a precursor. Certainly, the liver is not the sole organ in which cholesterol biosynthesis occurs. However,

TABLE 2
*Specific Radioactivities of Cholesterol of the Livers and Adipose Tissues
1 Hour after Intraperitoneal Injection of $1\text{-}^{14}\text{C}$ -Acetate*

Experimental group	Addition	Specific radioactivity	
		Liver cholesterol	Adipose tissue cholesterol
<i>cpm/mg</i>			
A	B vitamins	15,500	14,700
B	B vitamins + myoinositol	7,300	23,700
C	B vitamins + choline	10,000	14,500
D	B vitamins + myoinositol + choline	9,000	25,000

FIG. 1 *Changes in Specific Radioactivities of the Liver and Adipose Tissue Cholesterol with Time*

Rats of group A (stunted rats were given only B vitamins for a week) were injected with $15\text{ }\mu\text{Ci/rat}$ of $1\text{-}^{14}\text{C}$ -acetate and after certain times were sacrificed by decapitation. Specific radioactivities of cholesterol fractions of the liver and adipose tissue were measured as described in the text.

○, liver cholesterol; ●, adipose tissue cholesterol

FIG. 2 *Changes in Specific Radioactivities of the Liver and Adipose Tissue Cholesterol with Time*

Rats of group B (stunted rats were given B vitamins plus myoinositol for a week) were injected with $15\text{ }\mu\text{Ci}$ of $1\text{-}^{14}\text{C}$ -acetate and after certain times were sacrificed by decapitation. Specific radioactivities of cholesterol fractions of the liver and adipose tissue were measured as described in the text.

○, liver cholesterol; ●, adipose tissue cholesterol

Photograph 1. *Microscopic Observation of Liver Slides* ($\times 100$)

I: Liver of a normal rat; II: Liver of a stunted rat (a rat fed on a low protein, high carbohydrate diet with no B-vitamins, fats and cholesterol); III: Liver of a stunted rat given B vitamins for a week (group A); IV: Liver of a stunted rat given B vitamins + myoinositol for a week (group B); V: Liver of a stunted rat given B vitamins + choline for a week (group C); VI: Liver of a stunted rat given B vitamins + myoinositol + choline for a week (group D)

since most of the endogeneous cholesterol originated from the liver (10), it would be possible to assume that all the ^{14}C -labeled cholesterol in the liver and other tissues is synthesized from injected $1\text{-}^{14}\text{C}$ -acetate in this organ. Rats were administered intraperitoneally with $1\text{-}^{14}\text{C}$ -acetate, and the time course of its incorporation into cholesterol of the liver and adipose tissue was investigated.

FIG. 3 *Changes in Specific Radioactivities of the Liver and Adipose Tissue Cholesterol with Time*

Rats of group C (stunted rats were given B vitamins plus choline chloride for a week) were injected with 15 μ Ci/rat of 1-¹⁴C-acetate and after certain times were sacrificed by decapitation. Specific radioactivities of cholesterol fractions of the liver and adipose tissue were measured as described in the text. ○, liver cholesterol; ●, adipose tissue cholesterol

FIG. 4 *Changes in Specific Radioactivities of the Liver and Adipose Tissue Cholesterol with Time*

Rats of group D (stunted rats were given B vitamins + myoinositol + choline for a week) were injected with 15 μ Ci/rat of 1-¹⁴C-acetate and after certain times were sacrificed by decapitation. Specific radioactivities of cholesterol fractions of the liver and adipose tissue were measured as described in the text. ○, liver cholesterol; ●, adipose tissue cholesterol

The specific radioactivity of cholesterol from the liver and adipose tissue in each experimental group 1 hour after the injection is shown in Table 2. Higher specific radioactivities were found in the livers of animals given supplements containing no myoinositol (group A and C) than in those of animals given supplements containing myoinositol (group B and D). In adipose tissues, however, the reverse was the case; higher specific radioactivities were found in animals given myoinositol. This suggests that hepatic cholesterol becomes mobilized when rats suffered from fatty liver have been cured with myoinositol, if it can be assumed that ¹⁴C-cholesterol found in adipose tissues is synthesized from 1-¹⁴C-acetate in the liver.

In the case of animals suffering from fatty liver (group A), a change in cholesterol metabolism is suggested by the maintenance for a long time of a high level of ¹⁴C-labeled cholesterol in the liver. As shown in Fig. 1, higher specific activities were found in hepatic cholesterol than in adipose tissue cholesterol, during 24 hours after the injection.

On the other hand, specific activities of hepatic cholesterol rapidly decreased to a level as low as that found in adipose tissue, when rats were administered with myoinositol alone (group B, Fig. 2) or with choline together with myoinositol (group D, Fig. 4).

The above results may indicate that some prevention of cholesterol transport from the liver to the other tissues such as adipose tissue occurs when rats are suffering from fatty liver and that it disappears when rats are cured by administering a lipotropic factor, such as myoinositol.

When rats suffering from fatty liver were given choline alone, a somewhat different time course pattern was obtained (Fig. 3). This fact suggests a possibility that the action of choline alone on fatty liver is somewhat different from that of myoinositol alone.

DISCUSSION

Various causes have been known for the promotion of fat infiltration in the liver. Since the experimental fatty liver shown in the present study is considered to be the extreme example of this type of fatty liver, medicines which can aid recovery should have a wide usefulness for medical purposes.

Present results indicated that rats suffering from fatty liver as a result of nutritional imbalances were considerably aggravated by improving the vitamin balances. This fact strongly suggests that the supply of vitamins is not always effective for the improvement of vitamin deficiency, especially when animals are suffering from fatty liver as a result of vitamin deficiency. However, myoinositol as well as choline were found to be effective in the removal of fat from fatty liver caused by nutritional imbalances, as already clarified by many workers.

As reported in Table 1, liver lipids are considerably increased when rats fed on depleted diets with no B vitamins are furnished with B vitamin complex. Probably, livers of stunted animals may have sustained a harmful effect on their lipid transport system, so that fats and cholesterol newly synthesized after daily doses of B vitamins tend to accumulate there.

This was partly tested by tracing the movement of ^{14}C -labeled cholesterol synthesized principally in the liver after an *in vivo* injection of $1\text{-}^{14}\text{C}$ -acetate. The movement of cholesterol from the liver to adipose tissue of rats suffering from fatty liver was found to be considerably slower than that of rats cured with myoinositol. It took a much longer time to reach an equilibrium state in which specific radioactivities of hepatic- and adipose tissue-cholesterol are at the same level. Such a delay in cholesterol transport from the liver could be prevented on administering myoinositol.

In this connection, it may be valuable to note here that deposited fats in the liver appear to be removed from the environment of the central vein of each lobule, when rats suffering from fatty liver are administered with myoinositol. This fact suggests that the main role of myoinositol for the recovery of fatty liver is its beneficial effect on the movement of lipids from the liver to other tissues probably through the central vein.

ACKNOWLEDGEMENT

Authors are indebted to Prof. H. Tauchi of Pathological Department, for his helpful advice on histological experiments.

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