STUDIES ON THE METABOLISM OF α -LIPOIC ACID IN CHILDHOOD

I. EFFECT OF PROTEIN AND AMINO ACID COMPOSITION IN THE DIET ON THE BIOSYNTHESIS OF PROTEIN-BOUND LIPOIC ACID

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Reed (1) suggested that lipoic acid possessed the activity of coenzyme for oxidative decarboxylation of pyruvic acid and of α -ketoglutaric acid, and also isolated the substance later. Thereafter, lipoic acid has been included in vitamin B group, and has been widely used in the clinical fields. In spite of an increasing demand for clinical use, the chemical nature of the active form of lipoic acid, its activating mechanism and the accurate method for quantitative determination of the acid in biological material have not been established yet.

For utilization as the coenzyme in the living body, lipoic acid must be taken up into the apoenzyme protein concerning oxidative decarboxylation of pyruvic acid or α -ketoglutaric acid at first. Therefore, the incorporation of the acid into the apoenzyme protein is considered to be the most important premise.

Though, lipoic acid-protein complex has thus far been isolated from bacterial extract for the study of the mode of action of the acid as the coenzyme, Nose and Mori (2) demonstrated in the experiments with rats that the mitochondrial fraction contained 50 to 70 % of the acid in hepatic cells and that the acid was combined with protein molecule in mitochondria. In addition, S³⁵-labeled lipoic acid was incorporated into the mitochondrial protein fraction in the presence of ATP, MgCl₂ and succinic acid as cofactors *in vitro*.

The present studies were undertaken in order to observe the utilization of lipoic acid under various situations to test the incorporation of the acid into the mitochondrial protein fraction of rat liver.

EXPERIMENTAL

1. Assay Method of Combined S³⁵-Lipoic Acid

The livers of young male albino rats of Wistar strain, weighing 50 g, were removed and the mitochondrial fraction was isolated by Schneider-Hogeboom's method (3) and was incubated at 37°. It was added to the reaction system containing

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S³⁵-lipoic acid, succinic acid, ATP and MgCl₂. After 30 minutes of incubation, the protein fraction was precipitated with 10 % trichloroacetic acid, and free lipoic acid was extracted with acetone from the precipitates. The free lipoic acid fraction was hydrolyzed with 6 N H₂SO₄, and the S³⁵-lipoic acid was extracted with chloroform and the radioactivity was measured by a gas flow counter (Table 1).

TABLE 1
Procedure

1. Isolation of mitochondria	
2. Reaction (total volume 4.0 ml)	
Rat liver mitochondria suspension	1 ml
S ³⁵ -lipoic acid	50 μg
ATP and MgCl ₂	each 50 μmoles
Tris buffer (pH 7.4)	50 μmoles
Succinate	50 μmoles
→ 37.0°, 30 min incubation	
→ precipitation with 10 % trichloroacetic acid	
3. Washing with acetone	
4. Acid hydrolysis	
5. Extraction of S ³⁵ -lipoic acid	
6. Measurement of radioactivity	

Having fed the rats on a settled diet for 2 weeks, the animals were injected with S³⁵-lipoic acid intraperitoneally and the radioactivity of protein-bound lipoic acid was determined by the same procedure as in *in vitro* experiments, and the values were calculated as the radioactivity per mg nitrogen of the mitochondrial fraction of the liver.

2. *Test for the Effect of the Protein and Amino Acid Compositions of the Diets of Rats on the Biosynthesis of Protein-Bound Lipoic Acid*

Effect of Low Protein Diet — Young rats were divided into 2 groups: (a) low protein diet group (Group 2) was fed on the synthetic diet including 5 % casein, and (b) the standard diet group (Group 1) fed on the diet including 18 % casein as a protein source. The compositions of diets of both groups are given in Table 2.

Effect of Amino Acid Balance in the Diet — As the protein source the synthetic diet containing corn protein, zein, which was almost devoid of lysine as well as tryptophan in the amino acid composition, was used. They were divided

TABLE 2
Composition of Casein Diet

	Group of rats	
	1	2
	<i>per cent</i>	
Casein	18	5
McCormall salt mixture		4
Corn oil		5
Vitamin mixture		2
Starch	71	84

into three groups:

- (a) Tryptophan-deficient group: Lysine alone was added to the basic diet.
 (b) Lysine-deficient group: Tryptophan alone was added. (c) Control group: Lysine and tryptophan were added to the basal diet. (Table 3)

TABLE 3
Composition of Zein Diet

	Group of rats		
	1	2	3
	<i>per cent</i>		
Zein		18	
McCormall salt mixture		4	
Corn oil		5	
Vitamin mixture		2	
Starch	70.3	70.8	70.5
L-Lysine	0.5	—	0.5
L-Tryptophan	0.2	0.2	—

TABLE 4
Composition of Polished Rice Diet

	Group of rats						
	1	2	3	4	5	6	7
	<i>per cent</i>						
Polished rice	89.0	86.56	87.34	86.93	86.67	86.79	86.83
McCormall salt mix.				4			
Corn oil				5			
Vitamin mixture				2			
L-Lysine	—	0.78	—	0.78	0.78	0.78	0.78
L-Methionine	—	0.37	0.37	—	0.37	0.37	0.37
L-Tryptophan	—	0.11	0.11	0.11	—	0.11	0.11
L-Threonine	—	0.23	0.23	0.23	0.23	—	0.23
L-Phenylalanine	—	0.27	0.27	0.27	0.27	0.27	—
L-Valine	—	0.26	0.26	0.26	0.26	0.26	0.26
L-Leucine	—	0.27	0.27	0.27	0.27	0.27	0.27
L-Isoleucine	—	0.15	0.15	0.15	0.15	0.15	0.15

Effect of Other Essential Amino Acids in the Diet—Polished rice containing low protein, having the composition of amino acids different from casein, was used as the protein source. As the standard diet of polished rice group, all the essential amino acids for rats were added to polished rice to give the same amino acid composition as in casein according to Rose (5). For example, lysine-deficient diet was identical with the standard diet except that lysine alone had been excluded. The composition of the diet for each group is given in Table 4.

Experimental procedures were exactly the same as previously described.

RESULTS

1. The Effect of Low Protein Diet on the Biosynthesis of Protein-bound Lipoic Acid

The incorporation of S³⁵-lipoic acid into the total mitochondrial protein frac-

tion of the liver in a low protein diet group (Group 2) was lower than that of the standard diet group (Group 1), dependent on poor body weight gain and limited increase of the liver weight (Fig. 1). However, the incorporation per mg nitrogen of liver mitochondria of group 2 was unexpectedly higher than in Group 1 (Fig. 2). The same was also observed in the *in vivo* experiments in which S^{35} -lipoic acid was injected intraperitoneally to the rats fed on each experimental diet for 10 days (Fig. 3).

These results suggest that the protein required for the activation of lipoic acid does not easily lower its level under low protein diet as in other proteins in the liver, maintaining the normal level for a relatively long period. It is also concluded that the depleted formation of proteins, apo- α -keto acid oxidase and other protein of lipoic acid activating enzyme, was in slighter degree than that of other proteins in the liver. These tendencies are quite different from the fluctuations of xanthine oxidase and choline oxidase activities in the liver under the same conditions as were previously observed (6).

FIG. 1 *Incorporation of S^{35} -Lipoic Acid into Hepatic Mitochondrial Protein (casein diet group)*
—, group 1; ---, group 2

FIG. 2 *Incorporation of S^{35} -Lipoic Acid into Liver Mitochondrial Proteins (casein diet group)*
—, group 1; ---, group 2.

FIG. 3 *Incorporation of S^{35} -Lipoic Acid into Liver Mitochondrial proteins after Injection of the Labelled Acid (casein diet group)* ○—○, 5% casein diet group injected with 20 mg lipoic acid per kg; ○---○, 5% casein diet group injected with 10 mg lipoic acid per kg; ●—●, 18% casein diet group injected with 20 mg lipoic acid per kg; ●---●, 18% casein diet group injected with 10 mg lipoic acid per kg.

2. The Effect of Amino Acid Balance on the Biosynthesis of Protein-bound Lipoic Acid in the Zein Diet Groups

FIG. 4 *Incorporation of S^{35} -Lipoic Acid into Liver Mitochondrial Proteins (zein diet group)*

FIG. 5 *Incorporation of S^{35} -Lipoic Acid into Liver Mitochondrial Protein after Injection of the Labelled Acid (zein diet group)* —, group 1; ---, group 2; ···, group 3

The lipoic acid incorporation into the liver mitochondrial protein per mg nitrogen was decreased in the rats fed on a zein diet deficient in tryptophan or lysine. Especially in tryptophan-deficient group (Group 3), the depleted formation was more distinct than in lysine-deficient group (Group 2) (Fig. 4). These facts were also demonstrated by the *in vivo* experiments performed by injecting S^{35} -lipoic acid intraperitoneally into the rats fed on such deficient diet for 10 days (Fig. 5).

3. The Effect of Amino Acid Balance on the Biosynthesis of Protein-bound Lipoic Acid in the Polished Rice Diet Group

The activity of protein-bound lipoic acid formation was shown to be most distinctly depressed in the group fed on polished rice. The lysine-, tryptophan- or threonine-deficient group revealed a slighter defect than in the polished rice group, though incorporation of lipoic acid was markedly depressed in all four groups. On the contrary, the formation of protein-bound lipoic acid in the standard diet group of polished rice was highest. No significant difference was seen between the methionine- or phenylalanine-deficient group and the standard diet group (Figs. 6 and 7).

The order of body weight gain in each group is as follows. Standard polished rice group or methionine-deficient group > tryptophan- or phenylalanine-deficient

FIG. 6 Incorporation of S^{35} -Lipoic Acid into Liver Mitochondrial Proteins (rice diet group)

FIG. 7 Incorporation of S^{35} -Lipoic Acid into Liver Mitochondrial Proteins after Injection of the Labelled Acid (rice diet group)

—, group 1; --, group 2; ···, group 4, ---, group 6.

group > lysine- or threonine-deficient group > polished rice group. This order was not proportional to that of the activity of protein-bound lipoic acid formation.

In each experimental group the S^{35} -lipoic acid incorporation into the total mitochondrial protein fraction was almost in conformity with the incorporation per mg nitrogen of the liver mitochondrial protein.

DISCUSSION

From the above findings, it was clearly shown that the biosynthesis of protein-bound lipoic acid in the liver mitochondria was influenced by the protein and amino acid composition of the diet. Several kinds of protein are considered to be related with protein-bound lipoic acid formation. The apopyruvate oxidase, apo- α -ketoglutarate oxidase and the enzyme protein involving these reactions are considered to be the proteins related to the formation of protein-bound lipoic acid. Reed indicated the activating reaction as follows:

If these reactions are influenced by protein and amino acid composition of the diet, it is assumed that the production of the apoenzyme or other enzyme protein involved in the activating reaction is affected.

When the animal is fed on a low protein diet, it is generally considered that the proteins constituting the body tissues and enzymes will decrease in the same fashion. But the degree of decrease is quite variable, for example, Elvehjem (7) and Nakamura (6) indicated that the activities of xanthine oxidase and choline oxidase were most remarkably decreased following the low protein diet because their syntheses are very susceptible to the protein composition of the diet. The specific activities of these enzymes per mg nitrogen of the liver were distinctly low and the total activities in the whole tissues were also very low. These enzymes were more susceptible to the low protein diet than any other proteins in the liver.

However, the specific activity of lipoic-activating reaction showed higher value in the low protein group than that in the standard diet group. Therefore it is concluded that the depleted formation of the protein related to lipoic acid activating system, apo- α -keto acid oxidase and other protein involved in lipoic acid activation, is slighter than that of other liver proteins. In other words, the former has a stronger resistance to protein deficiency.

The incorporation of the administered lipoic acid into liver mitochondrial protein showed a peak after a short period, returning to normal after 2 to 6 hours. This information seems to be a valuable aid for clinical administration of lipoic acid.

On the contrary, the effect of dietary deficiency of amino acid, such as lysine, tryptophan and threonine, on the specific activity of activating lipoic acid is very prominent. Reed (8, 9) demonstrated that lipoic acid is bound with protein through amide-linkage with the α -amino group of the lysine residue of apopyruvate oxidase or apo- α -ketoglutarate oxidase. Therefore, it is expected that the formation of protein-bound lipoic acid is depressed in the lysine-deficient group, but it was also

shown from a series of our experiments that the formation of protein-bound lipoic acid in the tryptophan- or threonine-deficient group was more strongly depressed than in the lysine-deficient group. This suggests the importance of not only the amino acid linked with lipoic acid, but also the amino acids closely related with the formation of apoenzyme in lipoic acid-activating system.

At all events, in order to obtain an excellent utilization of lipoic acid as the coenzyme, an adequate qualitative and quantitative balance of proteins and amino acids in the diet must be maintained.

Although the dramatic effects of lipoic acid on the treatment of hepatitis, hepatic coma, dyspeptic intoxication, and cyclic vomiting, *etc.* have already been reported (10), it is necessary to direct our attention to the balance of proteins and amino acids to be administered in order to promote the effect of lipoic acid under such pathological conditions.

SUMMARY

The effects of the protein and amino acid composition in the diet on the biosynthesis of protein-bound lipoic acid, which is considered to be the active form of lipoic acid for oxidative decarboxylation of α -keto acids, such as pyruvic acid and α -ketoglutaric acid, were observed and the following findings were observed.

1. Decreased incorporation of lipoic acid into the total mitochondrial protein of the liver was revealed in the low protein group.
2. The incorporation of the acid per mg nitrogen of liver mitochondria of the low protein group was unexpectedly higher than that in the standard diet group.
3. In the lysine-, tryptophan- or threonine-deficient group, the incorporation of lipoic acid into the mitochondrial proteins was decreased, but in the methionine- or phenylalanine-deficient group, almost the same incorporation was observed as the standard diet group.

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