STUDIES ON THE PROMOTING FACTORS OF GALACTOSE AND LACTOSE METABOLISM IN THE WHEY

I. THE EFFECTS OF THE PROMOTING FACTORS ON THE GALACTOSE AND LACTOSE METABOLISM IN THE ACTIVITY OF THE LOEIO ENZYME SYSTEM IN THE CATARACT RAT

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Lactose exists only in milk as a product of the mammary gland and its considerable amount is found in the milk of many animals except in that of ocean animals, such as whales and seals(1). Lactose is suggested to play an important role for the biosynthesis of galactocerebroside (2-4) (Fig. 1).

On the other hand, lactose added to milk has an undesirable character. First, there are many reports on sucklings about the addition of lactose to the milk being deleterious. Second, many clinicians have experimented with congenital or acquired intolerances for lactose ingestion. Third, it has been reported by Tamura (5) that dyspepsia with diarrhoea in suckling is induced by the accumulation of galactose-1-phosphate, the inhibition of lactose activity in the intestinal wall and an acceleration of permeability of the intestine.

Riggs and Beaty (6) reported that animals, which are fed on a hyper-lactose diet, are arrested in their growth, and some disorder of the intestine, especially diarrhoea, occurs

FIG. 1. Biosynthetic pathway of cerebroside from galactose and glucose.

in mammals and birds. Mitchell and Day (8) found that the cataract occurs in the rat. Takuma and Takayama (9) and Tamura and Ogihara (10) also observed that cataract and a disorder of nutrition of rat caused by lactose are prevented by an addition of whey in the diet (Fig. 2).

The metabolism of lactose mentioned by Bihler and Crane (11) is as follows: lactose was decomposed to galactose and glucose, and turned in cytoplasm with sodium ion. Then, glucose was available in glycolytic or non-glycolytic metabolism, and galactose was metabolized through the Leloir enzyme system (1) (Fig. 3).

Now, we can take the view that the inhibition of enzymes occurred in the Leloir way in the above-mentioned disease of sucklings and rats. The authors studied the activity of the Leloir enzyme system in rats fed on a hyperlactose or a hypergalactose diet, and the effect of whey including the promoting factor of lactose or galactose metabolism upon this system.

EXPERIMENTAL METHODS AND MATERIALS

Experimental animals used in these studies were young albino rats of Wistar strain (21 days after birth with a body weight of 35–45 g). This colony was kept enclosed from 1950. The rats were raised in a special cage of stainless steel with a wire bottom. The rats were fed on an experimental diet for 10–15 weeks.

Experimental diet: It is shown in Table 1.

The measurement of activity of each enzyme was done as follows:

1. ATP: D-galactose-1-phosphotransferase (2.7.1.6) activity was done as follows:

Reagents—

0.01 galactose, 0.2 M MgCl_2 , 1 M NaF, 0.2 M Tris-HCl buffer (pH 7.7), 0.02 M ATP, 5% ZnSO_4 , 0.3 N $\text{Ba}(\text{OH})_2$.

Incubation—

The system contained, in addition to

hyper-lactose diet

1 2 3 4 5 6 7 8 9 10 (week)

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whey powder

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● cataract
+ death

FIG. 2. Influence of hyper-lactose diet on the occurrence of death or cataract in rats.

- ← Transformation in D-glucose and degradation of galactose
 ← Biosynthesis of D-galactose and D-galactoside
 ① ATP : D-galactose-1-phosphotransferase (2.7.1.6)
 ② UDP glucose : α-D-galactose-1-phosphate uridylyltransferase (2.7.7.12)
 ③ UDP galactose-4-epimerase (2.7.1.6)
 ④ UTP : α-D-glucose-1-phosphate uridylyltransferase (2.7.7.9)

FIG. 3. D-Galactose metabolism on the Leloir enzyme system.

TABLE 1. Composition of the experimental diet.

	Control	Cataract-diet	Whey-diet
Sucrose	55.2%		
Lactose		55.2%	55.2%
Wheat-powder	27.6	27.6	27.6
Casein	13.8	13.8	13.8
Salts*	3.4	3.4	3.4
Vitamins**	1.0	1.0	1.0
Whey			4 ml/day/rat

* vitamins (1.0 g)

vitamin A	2500 I.U.	nicotinic acid	10.0 mg
vitamin B ₁	1.0 mg	folic acid	0.5 mg
vitamin B ₂	1.5 mg	calcium pantoate	0.5 mg
vitamin B ₆	0.1 mg	vitamin C	37.5 mg
vitamin B ₁₂	1	vitamin D	200 I.U.

** salts McCollum salt mixture No. 185

1 ml of enzyme, 0.5 ml of galactose solution, 0.1 ml MgCl₂ solution and 0.8 ml of tris buffer, and then, was incubated with ATP, for 20 minutes at 37°C.

Deproteinization and determination—

The solution mixture was deproteinized with 3.0 ml of Ba(OH)₂ and 3.0 ml of ZnSO₄ and filtered. The glucose of the supernatant was determined by the method of Fujita and Iwatake (12).

2. UDP glucose: α -D-galactose-1-phosphate uridylyltransferase (2.7.7.12) activity was measured by means of the consumption test reported by Anderson *et al.* (13).

3. UDP glucose-4-epimerase (5.1.3.2) activity was measured by means of the consumption test reported by Isselbacher *et al.* (14).

4. UTP: α -D-glucose-1-phosphate uridylyltransferase was done by the modification of Kalckar and Anderson's method (15).

RESULTS

1. ATP: D-galactose-1-phosphotransferase (2.7.1.6) activity

In the activity of this enzyme, there were no differences among the control, the cataract and the whey group (Fig. 4).

2. UDP glucose: α -D-galactose-1-phosphate uridylyltransferase (2.7.7.12) activity

The activity of the enzyme in the cataract group fell to less than quarter compared that of the control group, but in the whey group it showed two times as much activity as the cataract group (Fig. 5).

3. UDP glucose-4-epimerase (5.1.3.2) activity

The activity of the enzyme evidently fell in the cataract group. But in the whey group there was no difference (Fig. 6).

4. UTP: α -D-glucose-1-phosphate uridylyltransferase (2.7.7.9) activity

This activity evidently fell in the cataract group, but only slightly in the whey group (Fig. 7).

FIG. 4. Changes of ATP: D-galactose-1-phosphotransferase activity.

FIG. 5. Changes of UDP glucose: α -D-galactose-1-phosphate uridylyltransferase activity.

FIG. 6. Changes of UDP glucose-4-epimerase activity.

FIG. 7. Changes of UTP: α -D-glucose-1-phosphate uridylyltransferase activity.

DISCUSSION

It was observed that some of the activities of enzymes in the Leloir enzyme system were disordered by the hyper-lactose or hyper-galactose diet, i.e. ATP: D-galactose-1-phosphotransferase (2.7.1.6), UDP glucose-4-epimerase (5.1.3.2) and UTP: α -D-glucose-1-phosphate uridylyltransferase (2.7.7.9). However, the inhibition of activities of these enzymes was improved by the addition of whey.

From the point of view of metabolism, excessive galactose from the lactose turned into galactose-1-phosphate, which availed little because of the disorder of the enzyme, and was accumulated in a body. Consequently, several disorders occurred in the living body by accumulated galactose-1-phosphate. An addition of whey improved these disorders. Then the authors ascertained that the existence of whey in milk activated again the enzyme dysfunctioned by hyper-lactose or hyper-galactose.

SUMMARY

1. In the Leloir enzyme system of rats fed on a hyperlactose diet, there was no change of the activity of ATP: D-galactose-1-phosphotransferase (2.7.1.6): as for UDP glucose-4-epimerase (5.1.3.2), UTP: α -D-glucose-1-phosphate uridylyltransferase (2.7.7.9) and UDP glucose: α -D-galactose-1-phosphate uridylyltransferase (2.7.7.12), we could see the inhibition of these activities.

2. The addition of whey improved the disordered activity of the Leloir enzyme system and prevented cataract and any bad condition from breaking out.

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