Riboflavine Concentration of the Biopsied Livers from Children with or without Nutritional Dystrophy

Studies on the Nutrition of Children in Hirosaki Area

(47th Report)

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The signs of riboflavine deficiency, such as angular stomatitis, cheilosis and swelling of the lingual papillae, were frequently found among children in Hirosaki Area, and these ariboflavinotic signs have been accepted as part manifestations of nutritional dystrophy¹⁾. In the preceding paper²⁾, Kagaya, one of us, reported that the blood level of riboflavine was, in general, lower in children with nutritional dystrophy than in those without it.

In the present investigation riboflavine concentration in the liver specimens biopsied from children with or without nutritional dystrophy will be dealt with.

EXPERIMENTAL

Method and Material

The liver biopsy was carried out in the same way as described in Sato's report³⁾. Adhering blood was blotted from the biopsied specimens, and 5–10 mg. of them were used for the estimation of riboflavine. The extraction of riboflavine from the liver tissues was performed according to Yagi's procedure⁴⁾ and riboflavine was determined by lumiflavin-fluorometric method^{5), 6)}.

The histological preparations of the biopsied liver and the description of their microscopic findings were done in the same way as described in Sato's report¹⁾.

The children subjected to this investigation were the patients admitted into our Clinic. 20 of these children had shown clinical signs of riboflavine deficiency. The rest, 10 in number, had been free from such symptoms.

Results and Comment

In the biopsied liver specimens from the 20 cases with nutritional dystrophy, the average for the total riboflavine concentration was $20.8 \pm 2.4 \gamma$ per gram, and that for the esterified form was $12.8 \pm 1.5 \gamma$ per gram, corresponding to 61.5% of the total riboflavine. In the biopsied liver specimens of the 10 children without nutritional dystrophy, the average for the total riboflavine was $28.2 \pm 7.1 \gamma$ per gram, and that for the esterified form was $16.6 \pm 3.2 \gamma$ per gram, being 58.9% of the total riboflavine (Cf. Table I and Fig. 1).

On the microscopic examination, the plant cell-like appearance of

TABLE I
Riboflavine Concentration in the Biopsied Liver Specimens
of Children with or without Ariboflavinotic Signs

No. of case	Name and sex	Age (years and months)	Method for nutrition in infancy	Signs of riboflavine deficiency	Liver palpable below the costal margin (cm.)	Clinical diagnosis
				Swelling of lingual papillae Angular stomatitis Cheilosis		
1	O.K. ♀	4.0	Mother's milk	## — —	4.5	Nutritional dystrophy
2	T.M. ♀	3.1	Dried milk	## — +	0.5	Mongolian idiocy
3	A.T. ♂	1.1	Dried milk + rice flour	## — +	3.0	Rickets
4	K.R. ♀	0.6	Rice flour + dried milk	## — —	3.0	Nutritional dystrophy
5	N.Y. ♂	0.2	Mother's milk + rice flour	++ — —	4.5	Enlargement of the liver
6	N.N. ♀	5.2	Mother's milk + cow's milk	++ + —	0.5	Nutritional dystrophy
7	H.S. ♀	0.6	Dried milk	— — —	0.5	Rhinopharyngitis
8	H.K. ♂	5.1	Mother's milk + rice flour	## + —	1.5	Mongolian idiocy
9	N.K. ♂	0.9	Mother's milk	— — —	0.5	Tuberculosis of hilar lymph nodes
10	S.Y. ♂	1.8	Dried milk	— — —	1.5	Diaphragmatic hernia
11	I.M. ♂	2.1	Dried milk	— — —	1.5	Rickets

12	M.F. ♀	4.0	Mother's milk + dried milk	## - -	7.0	Pick's polyserositis
13	Y.S. ♂	5.2	Mother's milk + dried milk	++ ++ -	3.0	Mongolian idiocy
14	N.K. ♀	5.1	Mother's milk	+ ++ ++	1.5	Tuberculosis
15	N.T. ♂	2.0	Mother's milk + dried milk	++ + ++	2.0	Nutritional dystrophy
16	S.K. ♂	3.9	Mother's milk	- - -	5.0	Enlargement of the liver
17	M.T. ♂	6.1	Mother's milk	++ - -	3.5	Nutritional dystrophy
18	M.S. ♂	2.6	Mother's milk	+ - +	2.0	Tuberculous meningitis + Nutritional dystrophy
19	K.Y. ♀	4.9	Mother's milk	## - -	3.0	Mongolian idiocy
20	M.K. ♀	0.6	Mother's milk	- - -	3.0	Rhinopharyngitis
21	K.Y. ♀	1.0	Mother's milk + rice flour	## - +	3.0	Atrophy
22	K.Y. ♀	1.1	Mother's milk + dried milk	## - -	6.0	Atrophy
23	K.Y. ♀	1.2	Dried milk	++ - -	4.0	Atrophy
24	K.T. ♀	0.4	Dried milk + rice flour	++ + +	3.0	Miliary tuberculosis
25	K.T. ♀	0.5	Dried milk	- - -	4.0	Miliary tuberculosis
26	K.T. ♀	0.8	Dried milk	- - -	3.0	Miliary tuberculosis
27	K.H. ♂	0.7	Dried milk + rice flour	- - -	2.0	Atrophy
28	K.H. ♂	0.8	Dried milk	- - -	3.0	Atrophy
29	S.I. ♂	1.4	Dried milk + rice flour	## - +	3.0	Nutritional dystrophy
30	S.I. ♂	1.4	Dried milk + rice flour	+ - +	1.0	Nutritional dystrophy

the hepatic cells³⁾ was found in 13 cases (Cases 1-4, 6, 8, 11, 13, 17, 19, 21, 26 and 27), and in these cases, the average for the total riboflavine concentration was $18.9 \pm 3.3 \gamma$ per gram and that for the esterified form was $11.7 \pm 2.3 \gamma$ per gram, being 61.9% of the total riboflavine. The plant cell-like appearance of the hepatic cells was not found in 12 cases (Cases 5, 7, 9, 10, 15, 16, 22, 24, 25, 28, 29 and 30), and in these cases, the average for the total riboflavine was $27.0 \pm 6.1 \gamma$ per gram and that for the esterified form was $16.7 \pm 2.5 \gamma$ per gram, being 61.9% of the total

TABLE IB
Riboflavine Concentration in the Biopsied Liver Specimens
of Children with or without Ariboflavinotic Signs

No. of case*	Histological changes of liver				Riboflavine concentration in the liver		
	Hepatic cells			Portal fibrosis	Total (r/g)	Esterified (r/g)	Ratio of esterified to total riboflavine (%)
	Plant cell-like appearance	Fatty infiltration	Atrophy				
1	++	—	—	—	12.9	12.5	96.9
2	+	—	—	—	20.0	8.3	41.5
3	+	+	—	+	13.6	9.8	72.0
4	+	—	—	++	21.0	10.9	51.9
5	—	—	—	++	15.5	14.0	90.3
6	++	++	+	—	21.2	15.7	74.1
7	—	—	—	—	18.9	17.7	93.7
8	+	—	—	—	18.9	14.8	78.3
9	—	—	—	—	30.0	26.6	88.7
10	—	—	—	—	28.1	18.8	66.9
11	++	—	—	—	20.1	10.8	53.7
12					21.1	12.8	60.7
13	+			+	19.1	15.2	79.6
14					32.3	15.6	48.3
15	—	—	++	—	24.3	12.0	49.4
16	—	—	++	++	22.4	14.5	64.7
17	+	—	—	—	12.4	8.1	65.3
18					23.2	11.0	47.4
19	++				18.1	16.2	89.4
20					28.2	16.0	56.7
21	++	—	—	—	15.6	3.8	24.4
22	—	—	—	—	27.3	14.4	52.7
23					22.5	13.0	57.8
24	—	—	—	—	24.5	15.9	64.9
25	—	—	—	—	27.1	20.0	73.8
26	+	—	—	+	33.0	11.2	33.9
27	++	—	—	—	20.3	15.1	74.3
28	—	—	—	—	53.6	15.2	28.4
29	—	—	—	—	30.9	15.9	51.5
30	—	—	—	—	22.2	15.9	71.6

* No. of case was the same as in Table I A.

riboflavine (Cf. Table I and Fig. 2). These results showed that the concentration of the riboflavine, in both total and esterified forms, tended to

Fig. 1. Riboflavin concentration in the biopsied liver specimens of children with or without ariboflavinotic signs.

Fig. 2. Riboflavin concentration in the biopsied liver specimens with or without plant cell-like appearance.

be higher in the biopsied livers without the plant cell-like appearance of the hepatic cells than in those with it (Cf. Fig. 1 and 2).

Sato³⁾ reported from the study of his own materials of liver biopsy that the plant cell-like appearance of the hepatic cells was observed in 22 cases out of 30 cases of the children with nutritional dystrophy and that this histopathological change might be due to high-carbohydrate-low-protein diet. As reported by Masuda *et al.*⁷⁾, in most of the inhabitants in this Area, about 60% of the caloric intake was provided with highly polished rice; vegetable and fish were scanty in variety, and eggs, milk and meat were scarcely taken. In a word, that is "a low protein-high-carbohydrate and B vitamin deficient diet."

Respecting the relationship between riboflavine metabolism and protein depletion, Sarett *et al.*⁸⁾ reported that concentration of riboflavine in the liver varied directly with the level of protein in the diet and was independent of the vitamin intake. This finding was later confirmed by Unna *et al.*⁹⁾, who showed that, in spite of the ingestion of liberal amount of riboflavine, the livers of rats maintained on a low protein diet were unable to retain riboflavine.

The clear relationship between a decreased concentration of riboflavine in the biopsied liver specimens and poor protein intake was observed from Table II, in which only the infants under 12 months were abstracted from the data of the present investigation. In Table II, the cases with ariboflavinotic signs were found in 5 (Cases 4, 5, 21, 24 and 27), all of which were those fed mainly on rice gruel with an addition of small amount of milk preparation or with poorly secreted human milk, and in 3 cases of which plant cell-like appearance of the hepatic cells was observed in the biopsied liver specimens. The concentration of riboflavine in the liver specimens, in both the total and esterified forms, tended to be lower in the infants fed mainly on rice gruel than in those fed on mother's milk or milk preparations exclusively.

SUMMARY AND CONCLUSIONS

The riboflavine concentration in the biopsied liver was lower in children with ariboflavinotic signs (as part manifestations of nutritional dystrophy) than in those without same.

Protein deficiency might be one of factors contributing to the development of ariboflavinosis in children in this District, because the riboflavine concentration in the biopsied liver specimens tended to be lower in those cases in which the plant cell-like appearance of hepatic cells was histologically demonstrated than in those without such histological changes.

TABLE II
Relation among Ariboflavinotic Signs, Riboflavine Concentration in the Biopsied Liver Specimens and Methods for Nutrition in Infants under 12 months

Method for nutrition	Mother's milk			Milk preparation			Mainly rice gruel with an addition of small amount of mother's milk or milk preparation		
Concentration of B ₂ in liver	No. of case	B ₂ in liver (γ/g)		No. of case	B ₂ in liver (γ/g)		No. of case	B ₂ in liver (γ/g)	
		Total	Esterified		Total	Esterified		Total	Esterified
	9	30.8	26.6	7	18.9	17.7	(4)*	21.0	10.9
	10	28.2	16.6	25	27.1	20.0	(5)	15.5	14.0
				26*	33.0	11.2	(21)*	15.6	3.8
				28	53.6	15.2	(24)	24.5	15.9
							(27)*	20.3	15.1
	Average	29.1 ± 14.9	21.3 ± 95.1	Average	33.1 ± 23.6	16.0 ± 6.1	Average	19.4 ± 4.6	11.9 ± 6.2

* The cases in which plant cell-like appearance of the hepatic cells was demonstrated by liver biopsy. () The cases with ariboflavinotic signs.

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