Formiminotransferase Deficiency Syndrome Associated with Megaloblastic Anemia Responsive to Pyridoxine or Folic Acid*

Tsuneo Arakawa, Tsunenobu Tamura, Ototaka Higashi, Kazuo Ohara,
Kaneo Tanno, Yoshiyuki Honda, Kuniaki Narisawa, Tasuke Konno,
Yoshiro Wada, Yasushi Sato and Takashi Mizuno

*Department of Pediatrics (Prof. Ts. Arakawa),
Faculty of Medicine, Tohoku University, Sendai*

A third case of formiminotransferase deficiency syndrome was described, which was associated with megaloblastic-pyridoxine-folic acid-responsive anemia of probably congenital origin. The occurrence of megaloblastic pyridoxine-folic acid-responsive anemia was not observed in two cases of formiminotransferase deficiency syndrome previously reported by us.

Formiminotransferase deficiency syndrome^{1,2} was firstly discovered by us as a new inborn error of folate metabolism which was characterized by 1) mental retardation, 2) hyperfolic acidemia, and 3) an excessive urinary excretion of formiminoglutamic acid (FIGLU) following an oral dose of L-histidine. A definite diagnosis of this syndrome was established by demonstrating a defective activity of formiminotransferase in the liver specimens obtained from patients in question.

The purpose of our present paper is to describe an infant with formiminotransferase deficiency syndrome associated with megaloblastic-pyridoxine-folic acid-responsive anemia probably of congenital origin.

REPORT OF CASE

Y.A., a three-month-old boy was admitted to the University Hospital on February 7, 1966, because of pallor developing soon after birth. The patient was the only child of healthy and nonconsanguineous parents. Pregnancy and delivery were uneventful and the birth weight was 2,600 g. Neonatal history revealed nothing abnormal except for pallor of the skin. His appetite was good and there was no history of diarrhea or vomiting.

At 15 days of life examination of peripheral blood was made at a local hospital and revealed that red cell count was $212 \times 10^4/\text{mm}^3$, hemoglobin 44% and white blood cell count $7,500/\text{mm}^3$. At that hospital from the 17th day of life onward,

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vitamin B₁₂, 500 γ daily, was given intramuscularly for a 4-day period, in addition to blood transfusions in a daily dose of 50 ml for consecutive 10 days. The anemia seemed to be improved following the therapy, but a relapse of anemia ensued two months later.

When 3 months old the patient was referred to the University Hospital for further evaluation of the anemia noted soon after birth.

Physical examination revealed a pale boy with a slightly distended abdomen. Auscultation on the lungs and heart gave no abnormality. The liver was palpable two finger breadths under the costal margin and soft in consistence. The spleen was not palpable. There was no lymphadenopathy. Deep tendon reflexes were normal and no pathologic reflexes were elicited. X-ray films of the chest, extremities and skull revealed no abnormality. The circumference of the head measured 39 cm, that of the chest 40 cm, body length 56 cm (normal 60.9 cm) and bodyweight 5.34 kg (normal 6.3 kg).

Laboratory findings

Urine was negative for bile, acetone and protein and for the FeCl₃ test, but slightly positive to Benedict's reaction on several occasions. The centrifuged sediments were clear except for 3 to 6 white cells per a high-power field.

Thin layer chromatography of urinary amino acids revealed no abnormality.

Liver function tests including CCFT, ZTT, GOT, GPT and alkaline phosphatase remained within normal limits on repeated examinations during his stay in the hospital.

Serum electrolytes were found to be normal except that serum iron levels were ranging from 135 to 202 γ /100 ml before pyridoxine therapy was instituted, and decreased to the levels from 46 γ /100 ml to 53 γ /100 ml after pyridoxine administration.

Serum phospholipids and total lipids were normal, but serum cholesterol was ranging from 64 mg/100 ml to 80 mg/100 ml before pyridoxine therapy and slightly increased to the levels of 80 mg/100 ml (Jan. 6, 1967) and of 102 mg/100 ml (May 4, 1967) after the pyridoxine therapy.

Serum levels of β -lipoprotein were estimated by Heiskell *et al.*'s method³ and were found to be of markedly lower values of 0.9 mm (Oct. 1966) and 0.9 mm (March 1967), as compared with those of normal infants (2.3–3.5 mm).

L-Tryptophan loading test: L-tryptophan, 100 mg per kg of bodyweight, was given and 24 hours' urine specimens were collected and estimated for urinary pyridone and xanthurenic acid, and subjected to paperchromatographic analysis of other tryptophan metabolites. Urinary pyridone⁴ was found to be 31 μ moles/24 hr/kg and 169 μ moles/24 hr/kg before and after the oral dose of L-tryptophan, respectively. Urinary excretion of xanthurenic acid⁵ was 4.6 μ moles/24 hr/kg and 2.7 μ moles/24 hr/kg, respectively, before and after the oral dose.

The findings obtained from the L-tryptophan loading test revealed no evidence suggestive of a disturbed tryptophan metabolism due to vitamin B₆ deficiency.

TABLE 1. Serum folate levels (*L. casei*) and urinary formiminoglutamic acid following an oral dose of *L-histidine monohydrochloride*, 0.33 g/kg in our own patient (Y.M.)

Date of examination	Age	Serum folate (<i>L. casei</i>) mγ/ml	Urinary formiminoglutamic acid following an oral dose of <i>L-histidine monohydrochloride</i> , 0.33 g/kg		Folic acid intake from milk preparations (γ per day)	Remarks
			μ moles/ml	μ moles/8 hours' urine		
1966						
Jan. 2m	2m	80	1.68	100	From Jan. 1st to Sept. 29th. 260-350γ per day	
Feb. 3m	3m	100	2.7	175		
March 8	5m	53				
April 19	6m	68				
May 12	9m	66				
Aug. 22	10m	57	0.64	86	Since Sept. 30th 50-90γ per day	Pyridoxal phosphate, 100 mg daily, was intramuscularly injected for consecutive 8 days (from Dec. 13, 1966 to Dec. 20) and 50 mg daily for 7 days (from Dec. 21 to Dec. 27)
Sept. 25						
Nov. 20		6.4				
1967						
Jan. 25	11m	4.4	7.2	906		Pyridoxal phosphate, 100 mg daily, was intramuscularly injected for consecutive 25 days (from June 10 to July 5)
Feb. 2	1m		13.4	1,742		
March 14	1y					
March 17	1y	2.3	4.6	598		
April 25	2m		5.1	1,123		
May 19	3m		7.4	1,288		Pteroylglutamic acid, 10 mg daily, was injected intravenously for consecutive 8 days (from July 7 to July 15, 1967)
May 28	4m		6.8	1,593		
June 7						
June 20			8.4	1,526		
June 25			7.6	875		
June 30			6.4	962	Since July 17, 200-300γ of folate per day	
July 5	1y 5m		16.1	2,093		
July 15			0.395	100		
July 26			0.585	220		

AICA loading test:⁶ Urinary excretion of AICA was found to be 0.13 mg/kg/day and 2.14 mg/kg/day before and after an oral dose of AICA, 20 mg/kg (June 1967), respectively. Urinary AICA after the oral dose was considered to be increased above the normal limit of 1.5 mg/kg/day.

No abnormality was found in any of glucose tolerance (June 1966), Moljodol (lipiodol) absorption (July 1966) and triiodothyronin uptake tests (July 1966).

Chromosomal analysis revealed a normal pattern in number and shape.

Histologic findings of the liver specimens obtained by surgical biopsy on Dec. 15, 1966 revealed hemosiderin deposits in hepatic cells and Kupffer's cells and round cell infiltration of slight degree in the portal tracts (cf. Fig. 1).

Fig. 1. Histologic findings of the liver of our own patient.

Urinary excretion of formiminoglutamic acid (FIGLU) after an oral dose of L-histidine monohydrochloride, 0.33 g per kg of bodyweight: Urinary excretion of FIGLU¹¹ following the oral dose of L-histidine was found to be increased, ranging from 0.64 to 2.7 μ moles per ml in spite of the presence of high serum folate levels over a period from January 1966 to September 1966. The increased excretion of FIGLU was more exaggerated during the period where serum folate turned to normal or subnormal levels after the patient had been placed on a low folate milk preparation (from September 29, 1966).

An administration of pyridoxal phosphate, 50–100 mg daily intramuscularly for consecutive 15 days, did not bring about any significant effect upon urinary excretion of FIGLU following an oral dose of histidine, showing urinary FIGLU ranging between 6.4 μ moles/ml (875 μ moles/8 hr urine) and 16.1 μ moles/ml (2,093 μ moles/8 hr urine). On the other hand, after an intravenous administration of

pteroylglutamic acid, 10 mg daily for consecutive 8 days, urinary FIGLU following the oral dose of histidine was found to be markedly reduced to values of 0.395μ mole/ml (100.7μ moles/8 hr urine) (July 15, 1967), and 0.585μ mole/ml (220.4μ moles/8 hr urine) (July 26, 1967), which were considered to be still above the upper limit of normal range (0.1μ mole/ml).¹²

Activities of tetrahydrofolate-dependent enzymes of the liver and erythrocytes: Liver specimens obtained by surgical biopsy were used for the assay of formiminotransferase, serine hydroxymethylase and N⁵ methyltetrahydrofolate transferase activities.

Erythrocyte lysate was used for the assay of N^{5,10} methylenetetrahydrofolate dehydrogenase, and formate activating enzyme activities.

Detailed procedures of each of these enzyme assays were the same as those described in previous papers of ours.^{13,14}

TABLE 2. *Formiminotransferase activity of the liver from our own patient and controls*

Subjects examined		Formiminotransferase activity of liver ($m\mu$ moles of N ^{5,10} methylenetetrahydrofolate per min per mg of protein)
Our own patient	Y.M. 1y. 2m. Formiminotransferase deficiency associated with megaloblastic pyridoxine-folic acid-responsive anemia	5.7
Control	K. U. 2 y. 3½m. N ⁵ methyltetrahydrofolate transferase deficiency	31.2
	S.K. 2 y 11 m. Mental retardation	24.8
	S. H. 1 y. 1 m. Hyperuricemia	30.2
	I.E. 2 y. 5 m. Mental retardation	18.1
	Y.T. 0 y 6 m. Mental retardation	25.6
	K. T. 0 y 7 m. Mental retardation	18.3
	A. B. 63 y. Cholelithiasis	29.8
	K.E. 60 y. Gastric cancer	25.2
	E.T. 60 y. Gastric cancer	17.3

As is shown in Tables 2–6, a marked decrease in formiminotransferase activity was observed on liver specimens obtained from our own patient, whereas other tetrahydrofolate dependent enzyme, such as serine hydroxymethylase, and N⁵ methyltetrahydrofolate-transferase of the liver specimens, and formate activating enzyme, and N^{5,10} methylenetetrahydrofolate dehydrogenase of erythrocytes did not differ in their activities from those of controls.

TABLE 3. *N⁵ methyltetrahydrofolate transferase activity of the liver from our own patient and controls*

Subjects examined	N ⁵ methyltetrahydrofolate transferase activity of liver (cpm of labeled methionine per hour per mg of protein)*
Y. M. ♂. 1y. 2m. Formiminotransferase deficiency associated with megaloblastic pyridoxine-folic acid-responsive anemia	2,094
A. B. ♂. 60 y. Gastric cancer	1,934
C.K. ♀. 0 y. 1 m. Diaphragmatic hernia	1,848

* 580 mμ moles of N⁵ methyltetrahydrofolate (sp. activity of 2,560 cpm/mμ moles) were used.

TABLE 4. *Serine-hydroxymethylase activity of the liver from our own patient and controls*

Subjects examined		Serine-hydroxymethylase activity of liver (mμ moles of N ^{5,10} methenyl-tetrahydrofolate per 60 min per mg of protein)
Our own patient	Y.M. ♂. 1 y. 2 m. Formiminotransferase deficiency associated with megaloblastic pyridoxine-responsive anemia	1,220
	K.U. ♂. 2 y. 4 m. N ⁵ methyltetrahydrofolate transferase deficiency	1,490
Control	U.M. ♀. 1 y. 10 m. Infantile spasm	1,340
	T.K. ♀. 0 y. 9 m. Mental retardation	1,360
	T.Y. ♀. 0 y. 7 m. Mental retardation	980
	K.T. ♂. 11 y. Choledochal cyst	750
	A.E. ♂. 60 y. Gastric cancer	880
	K.E. ♂. 61 y. Esophageal cancer	850
	S.A. ♂. 26 y. Polyp of stomach	1,320
	A.K. ♂. 29 y. Pancreatitis	1,460
	S.T. ♂. 40 y. Cholelithiasis	1,250
	U.E. ♂. 61 y. Gastric cancer	880
	K.U. ♂. 42 y. Peritonitis carcinomatosa	1,660
	O.T. ♀. 63 y. Cholelithiasis	1,980

TABLE 5. *Formate activating enzyme activity of erythrocytes from our own patient and controls*

Subjects examined		Formate activating enzyme activity of erythrocytes ($m\mu$ mole of $N^{5,10}$ methenyltetrahydrofolate per hour per mg of hemoglobin)
Our own patient	Y.M. ♂. 1 y. 2 m. Formiminotransferase deficiency associated with megaloblastic pyridoxine-folic acidresponsive anemia	4.5
Control	T.K. ♀. 1 y. 0 m. Mental retardation	6.3
	S.Y. ♂. 1 y. 0 m. Hyperuricemia	5.0
	T.O. ♂. 1 y. 0 m. Failure of growth	4.2
	U.M. ♀. 1 y. 0 m. Convulsions	6.0

TABLE 6. *$N^{5,10}$ methylenetetrahydrofolate dehydrogenase activity of erythrocytes from our own patient and controls*

Subjects examined		$N^{5,10}$ methylenetetrahydrofolate dehydrogenase activity ($m\mu$ mole of $N^{5,10}$ methenyltetrahydrofolate per hour per mg of hemoglobin)
Our own patient	Y.M. ♂. 1 y. 2 m. Formiminotransferase deficiency associated with megaloblastic pyridoxine-folic acidresponsive anemia	17.7
Control	T.O. ♂. 1 y. 0 m. Failure to thrive	15.2
	T.K. ♀. 1 y. 0 m. Mental retardation	18.7
	K.G. ♀. 12 y. Marfan's syndrome	21.3
	S.Y. ♂. 1 y. 0 m. Hyperuricemia	15.8
	U.M. ♀. 1 y. 0 m. Infantile spasm	6.0

Electroencephalograms taken at the age of 5 months revealed no abnormality, but those at the age of 1 year 5 months showed a low voltage in frontal areas.

Pneumoencephalography carried out at the age of 1 year 5 months revealed that Evans' ratios were 0.34 on the right and 0.30 on the left side and the third ventricle was 8 mm in diameter, and that there was air accumulation suggestive of diffuse cortical atrophy of frontal areas (cf. Fig. 2).

At the age of 1 year 8 months the patient could walk with support but speak only a few words.

Fig. 2. Pneumoencephalogram of our own patient showed that Evans' ratio was 0.34 on the right, 0.30 on the left side, the third ventricle being 8 mm in diameter. There was air accumulation suggestive of cortical atrophy.

Serum vitamin B₁₂ was estimated on March 1967 using *L. leichmannii* ATCC 4797 with a normal value of 0.93 mγ/ml.

Serum L. casei folate activity: Serum folate levels were found to be abnormally high, ranging from 57 mγ/ml to 100 mγ/ml during a period of from the early stage of his admission to September 1966, and fell down to normal and subnormal levels since November 20, 1966. This decrease in serum folate levels was considered to be due to a decrease in daily folate intake, because milk preparation which had been used until September 29, 1966 contained 38γ of folic acid per 100 ml, but that used after September 30, 1966 was far less in the amount of folate than the former, containing only 8γ per 100 ml (cf. Table 1).

Estimation of folic acid in milk preparations was carried out in the same way as described by Naiman and Oski¹⁰ except that samples were diluted up to 1:10 with 0.1M phosphate buffer of pH 6.1⁸ containing 500 mg of ascorbic acid per 100 ml, and were autoclaved at 121°C for 3 min⁸ or at 118°C for 10 min.⁹

Hematologic findings (cf. Table 7 and Fig. 3): After admission his anemia persisted and repeated blood transfusions were required to maintain his red cell counts appropriately, until pyridoxal phosphate was administered. The intramuscular injections of pyridoxal phosphate consisting of 100 mg daily for 8 days and 50 mg daily for next 7 days resulted in a remarkable increase in reticulocyte count and a gradual increase in erythrocyte count.

In bone marrow smears megaloblastoid cells and ringed sideroblasts were

Fig. 3. Effect of pyridoxine and folic acid administration upon hematologic findings of our own patient.

found on February 1, 1966, when the patient was 3 months old. After pyridoxine therapy, both megaloblastoid cells and ringed sideroblasts still persisted in the bone marrow smears examined on June 10, 1967 (cf. Table 7 and Fig. 3).

Folic acid was administered by intramuscular injections in a daily dose of 10 mg for consecutive 9 days, with a marked reticulocyte response (cf. Table 7 and Fig. 3). After the folic acid therapy, ringed sideroblasts decreased markedly in number (July 27, 1967), but megaloblastoid cells showed a slight decrease in number (cf. Table 7).

TABLE 7. *The hematological*

Date	30 Nov. 1965	14 Dec.	21 Dec.	11 Jan. 1966	7 Feb.	2 Mar.	16 May	4 July	27 Aug.	2 Oct.
Red cell count ($\times 10^4/\text{mm}^3$)	228	211	203	240	278	228	294	225	200	161
Hemoglobin (g/100 ml)	7.5	7.6	5.7	7.2		6.4	9.4	8	6.4	5.1
Hematocrit (%)	23		20	22	23	26	27	22	18	18
MCV (μ^3)	109		98	91	82	114	91	97	90	111.7
MCH (μg)	32	36	28	30		28	31	35	31	31.6
Reticulocytes (%)	19			10	10		1	7	1	39
White cell count (per mm^3)	7,500	7,100	3,000		10,800		3,500	9,800	4,200	
Basophils (%)					0		1	0	0	
Eosinophils (%)					1.0		3	2	1	
Lymphocytes (%)					66.5		67	54	85	
Monocytes (%)					3.5		5	7	2	
Neutrophils (%)					29.0		24	37	12	
I					28		52	14		
II					42		38	68		
III					28		6	14		
IV					0		4	4		
V					2		0	0		
Platelets ($\times 10^4/\text{mm}^3$)							3	54		
Bone marrow findings										
Megaloblastoid cells										
per 100 erythroblasts										
per 100 proerythroblasts										
Sideroblasts										
total, per 100 erythroblasts										
ringed sideroblasts, per 100 erythroblasts										
Total nucleated cell count, per mm^3										
M:E ratio										

DISCUSSION

The disease presented herein was diagnosed as a case of formiminotransferase deficiency syndrome, because of the presence of abnormal high levels of serum *L. casei* activity, abnormally large amount of urinary FIGLU following an oral

load of L-histidine and a marked decrease in formiminotransferase activity of the liver.

Up to date cases of formiminotransferase deficiency with a definite diagnosis were only two cases^{1,2} in our experience and the present case is the third of this syndrome where a defective activity of formiminotransferase of the liver was established definitely.

As compared with clinical manifestations of the previous two cases of formiminotransferase deficiency syndrome reported by us, the present case differed

data of the present case

14 Nov.	12 Dec.	19 Dec.	22 Dec.	9 Jan. 1967	4 Mar.	8 April	6 May	4 June	24 June	21 July	24 July	4 Aug.
237	336	300		275	330	406	405	497	444	370		407
7.8	10.8	9.2		9.2	10.8	14.0	13	17.6	14.6	12.2		13.1
21	36	31.5	30.5	30.0	34.0	42.0	41	49	48	36		44
88.6	107.1	105		109.0	103.0	103.4	101.2	98.5	108.1	97.3		109.1
32.9	32.1	30.6		33.4	32.7	34.4	32.0	35.4	32.8	32.9		32.5
5	20	73	102	136	64	69	50	32	40	82	107	44
7.400	5.000	4.700		5.600	4.300	6.000	9.000	5.200	7.200	7.700		
						1				0		
						2				1		
						56				68		
						10				3		
						31				28		
						30				23		
						30				55		
						36				22		
						4				0		
						0				0		
				Feb. 22 1967		June 10 1967				July 27 1967		
				12		17				4		
				54		50				52		
				89		96				76		
				26		32				1		
				30,000		98,000				28,000		
				1.3:1.0		1.0:1.2				1.0:1.5		

from the former two in that megaloblastic-pyridoxine-folic acid-responsive anemia appeared as one of prominent clinical manifestations and that mental retardation of the present case was much less in degree even though dilatation of cerebral ventricles and cortical atrophy were demonstrated in pneumoencephalograms.

In the present case of formiminotransferase deficiency a hematological

diagnosis was made of sideroblastic-megaloblastic and pyridoxine-folic acid-responsive anemia^{15,16} of probably congenital origin basing upon following findings:

1) An anemia was found soon after birth and both ringed-sideroblasts and megaloblastoid cells were found in bone marrow smears examined at the age of 3 months, 2) after pyridoxine administration, a remarkable rise in reticulocyte count followed by an increase in erythrocyte count ensued, but megaloblastoid cells and ringed-sideroblasts still persisted in bone marrow smears repeatedly examined thereafter, and 3) there was no increase in urinary excretion of xanthurenic acid following an oral loading of tryptophane which was carried out before pyridoxine administration was instituted.

A congenital occurrence of pyridoxine-responsive megaloblastic anemia does not seem to have appeared in the literature so far. In 1964 Horrigan and Harris¹⁷ published a detailed report of analysis of 62 cases of pyridoxine-responsive anemia, in which the cases with normoblastic erythropoiesis were found in 48 cases, those with undetermined bone marrow pictures in 4 cases and those with megaloblastic erythropoiesis in 10 cases. Among the 10 cases of megaloblastic erythropoiesis no case of congenital onset was recorded, whereas 3 cases of congenital onset were recorded among 48 cases with normoblastic erythropoiesis, and also one congenital onset was recorded among 4 cases with undetermined bone marrow findings.

At the present time it remains obscure whether the association of formiminotransferase deficiency with the development of sideroblastic-pyridoxine-responsive anemia is significant or only coincidental.

On assuming that formiminotransferase deficiency and sideroblastic-megaloblastic-pyridoxine responsive anemia observed in our own patient are of significant association, if any, with one another in their pathogenesis, the following two possibilities might be evaluated for an explanation of the association.

a) The first is that formiminotransferase deficiency is a primary congenital lesion of our own patient and that it leads to subsequent development of sideroblastic-megaloblastic pyridoxine-responsive anemia. This assumption is derived from the reason that a defective activity of formiminotransferase gives rise to an impaired utilization of folic acid, that is, 'functional deficiency'¹⁸ of folates available for hematopoiesis, thus leading to development of megaloblastic changes in the bone marrow, and the resulting impaired hematopoiesis induces an elevation in serum iron levels,^{19,20} the latter leading to an increase in incidence of sideroblasts in the bone marrow.²¹ As an evidence supporting this conception, it may be worthy to note that ringed-sideroblasts observed in the bone marrow smears were markedly reduced in number after the folic acid therapy was instituted in our own patient (cf. Table 7).

In our present case of formiminotransferase deficiency, a marked rise in reticulocyte count was observed after the pyridoxine administration. An explanation for this is that pyridoxine given in large doses exerts an accelerating effect, if any, upon the serine hydroxymethylase reaction, thus resulting in an increased

yielding of N^{5,10} methylenetetrahydrofolate available for hematopoiesis. In our case of formiminotransferase deficiency, however, serine hydroxymethylase activity of the liver was found to be normal (cf. Table 4).

As for the relationship between folate metabolism²³ and sideroblastic anemia, MacGibbon and Mollin¹⁶ made a detailed investigation upon 70 cases of sideroblastic anemia and concluded that folic acid deficiency, the diagnosis of which was based upon the results of serum folate assay and urinary FIGLU test, was found in many cases of sideroblastic anemia and that this folate deficiency resulted from an increased requirement of folic acid in many diseases associated with sideroblastic anemia.

b) The second is such a possibility that formation and/or function of formiminotransferase system may be dependent on pyridoxine in a similar way, as the anemia of our own patient is pyridoxine responsive. This speculation may arise from the results reported by Vitale *et al.*²² on one hand and those reported by Harris²³ on the other; in 1966, Vitale *et al.* found that formiminotransferase of the liver was markedly decreased in rats with iron deficiency and suggested a possibility of requirement of iron for function and/or formation of formiminotransferase system; in 1964, Harris investigated the role of pyridoxine in heme biosynthesis of two adult cases of megaloblastic-pyridoxine-responsive anemia and concluded that pyridoxine was necessary for release of heme from mitochondria of reticulocytes taken from these patients before pyridoxine therapy was instituted.

In our own patient, both pyridoxine responsive anemia and formiminotransferase deficiency were demonstrated from early infancy, so a question may arise whether or not an impaired utilization of iron due to pyridoxine dependency is a causative factor common to both defective activity of formiminotransferase and pyridoxine-responsive anemia.

This possibility turned to be unlikely from the experimental result recently obtained by Tamura¹⁴ of our Laboratory that formiminotransferase activity of the liver was not decreased in mice with pyridoxine deficiency induced by desoxypyridoxine or dietary deficiency.

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