Formiminotransferase Activity in Liver of Vitamin B₁₂ Deficiency

By

Kazuo Ohara, Ryo Chiba, Yoshiro Ito and
Yoshihiko Takahashi

*From the Department of Pediatrics, Faculty of Medicine, Tohoku
University, Sendai; Director: Prof. Ts. A r a k a w a*

(Received for publication, March 25, 1964)

Formiminotransferase activity in the liver was only slightly decreased when rats were made severely vitamin B₁₂ deficient by feeding with vitamin B₁₂-free diet for 30 days or more consecutive to the operation of forming a self-filling diverticulum of the small intestine.

In a preceding paper¹⁾ from our Laboratory, an infant with an abnormally high level of "serum folic acid activity" was reported, and it was confirmed that this abnormality in folic acid metabolism was due to a congenital defect in formiminotransferase of the liver.

In our recent investigation²⁾, it was found that in malnourished children, who showed ariboflavinotic signs and relatively low levels of serum vitamin B₁₂, abnormally high levels of folic acid activity in serum developed after oral dose of folic acid. At present time this abnormal finding remains unsolved for us.

In 1962 Knowles and Prankard³⁾ suggested that there was a disturbance in folic acid metabolism — a possible decrease in formiminotransferase — in patients with pernicious anemia or with megaloblastic anemia due to vitamin B₁₂ deficiency.

In these respects we have made present investigation in order to study the interrelationship between vitamin B₁₂ deficiency and formiminotransferase activity of the liver of rats.

METHODS AND MATERIALS

Each of the Wister rats, weighing from 30 to 50 g, was maintained in an individual cage. Diet was given *ad libitum*. Standard diet was of the composition as shown in Table I. Vitamin B₁₂-free diet was of the same composition

TABLE I. Composition of Standard Diet for Rats

Composition	%
Casein	20
Corn starch	40
Sucrose	27
Oil {soy bean oil 4 cod-liver oil 1	8
Salt mixture*	4
Vitamin mixture**	0.85
Cholin chloride	0.15

* Salt mixture: CaCO_3 , 29.29%; $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, 0.43%; KH_2PO_4 , 34.31%; NaCl , 25.06%; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 9.98%; $\text{Fe}(\text{C}_6\text{H}_5\text{O}_7) \cdot 6\text{H}_2\text{O}$, 0.623%; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.156%; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.121%; ZnCl_2 , 0.02%; KI , 0.0005%; and $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 0.0025%

** Vitamin mixture: Thiamin chloride, 0.059%; riboflavin, 0.059%; Ca-pantothenate, 0.235%; niacin, 0.294%; pyridoxine chloride, 0.029%; menadion, 0.006%; biotin, 0.001%; folic acid, 0.002%; inositol, 1.176%; ascorbic acid, 0.588%; vitamin B_{12} , 0.0002%; and lactose 97.551%

as that of the standard diet except for omission of vitamin B_{12} from the vitamin mixture. The diet used in this experimentation was available from the Tanabe Hissu-amino-san Kenkyu Kikin, Osaka, Japan.

Operation of "self-filling diverticulum" of the small intestine: Diverticulum of the small intestine was formed according to the method described by Strauss *et al.*⁴⁾ The small intestine was transected 40 cm from the ileocecal valve. The cut end of the distal intestine was anastomosed to the proximal intestine 8 cm from the cut end of the cephalad segment. The end of the proximal segment was tied off with a purse-string suture. Thus the normal direction of peristalsis in the intestinal segment of which a diverticulum was made, was towards the tied off end.

Rats were divided into 3 groups (Groups I, II and III). Group I consisted of 4 rats (Nos. 1, 7, 23 and 29) which were given vitamin B_{12} -free diet immediately following the operation for the self-filling diverticulum.

Group II included 6 rats (Nos. 8, 11, 14, 27, 28 and 40) which were given vitamin B_{12} -free diet without the operation.

Group III consisted of 6 rats (Nos. 9, 15, 21, 22, 25 and 30) which were given the standard diet without the operation.

Estimation of vitamin B_{12} contents in serum and liver was performed according to the method⁵⁾ adopted by Vitamin B Committee of Japanese Society of Vitaminology using *L. leichimannii* ATCC 4797.

Formiminotransferase activity of the liver was measured in the same way as described in our preceding paper¹⁾, using Tabor and Wyngarden's enzymatic method⁶⁾, and the activity was expressed as a difference of optical density between 420 m μ and 355 m μ read at one minute and/or 3 minutes after onset of the enzymatic reaction.

RESULTS AND DISCUSSION

Experiment I

Rats (Nos. 1 and 29) of Group I, which were given vitamin B₁₂-free diet for 30 days immediately following the operation for "self-filling diverticulum" of the small intestine. A rat (No. 14) of Group II was given vitamin B₁₂-free diet for 30 days, and a rat (No. 25) of Group III was control and given the standard diet.

As will be seen from Table II, serum vitamin B₁₂ contents of rats belonged to

TABLE II. Experiment I

Group	Nos. of rats	Serum vitamin B ₁₂ (m γ /ml)	Vitamin B ₁₂ content in the liver (m γ /g)	Formiminotransferase activity of the liver	
				Read at one minute	Read at 3 minutes
I (Self-filling diverticulum + B ₁₂ -free diet)	29	0.57	35	0.020	0.084
	1	0.48	107	0.024	0.049
II (B ₁₂ -free diet)	14	0.58	116	0.045	0.094
III (Control)	25	1.63	186	0.058	0.095

* Liver homogenates used were prepared so as to be of protein levels of 11 mg/ml. Formiminoglutamic acid in the reaction mixture was 0.2 micromole per tube.

Groups I and II were markedly reduced and amounted to about one-third of that of the control group, while vitamin B₁₂ contents in the livers were reduced in a less degree than those of serum, even though a considerable decrease in vitamin B₁₂ concentration was found in the livers of rats of Group I. It is worthy of notice that a decrease in formiminotransferase activity of the liver, when measured at one minute, was almost proportional to the decrease in vitamin B₁₂ concentration of the liver. But such a relationship between vitamin B₁₂ content in the liver and formiminotransferase activity as above mentioned, was no more found when formiminotransferase activity was measured at 3 minutes' reading.

Thus it might be assumed that a slight inhibition of an early stage of the formiminotransferase reaction was brought about by a decrease in vitamin B₁₂ content in the liver of rats, so far as the condition described in Experiment I was

concerned.

Experiment II

In this experiment, formiminoglutamic acid used in the reaction mixture was increased up to 1 micromole per tube, and influence of vitamin B₁₂ upon formiminotransferase activity was examined *in vitro* (cf. Table II).

Vitamin B₁₂ content in the liver of a rat of Group I (No. 23) was markedly reduced, but that of Group II (No. 11) showed no significant reduction from that of control (No. 15).

TABLE III. Experiment II

Group	Nos. of rats	Vitamin B ₁₂ content in the liver (mg/g)	Formiminotransferase activity of the liver (read at one minute)		
			Without addition of vitamin B ₁₂	With addition of vitamin B ₁₂	
				15mg/tube	150mg/tube
I*	7		0.096		
(Diverticulum + B ₁₂ -free diet)	23	45	0.130	0.119	0.118
II	8		0.180		
(B ₁₂ -free diet)	11	128	0.266		
III	15	150	0.140	0.124	0.112
(Control)	30		0.160		0.156

* Duration of feeding with vitamin B₁₂-free diet with or without self-filling diverticulum varied from 30 to 40 days.

** Liver homogenates were prepared so as to be of protein level of 9.5 mg/ml. Formiminoglutamic acid in the reaction mixture was 1 micromole per tube.

Respecting the formiminotransferase activity of the liver read at one minute of the enzymatic reaction, a considerable decrease was found in only one case (No. 7) out of 2 cases (Nos. 7 and 23) of Group I. This finding differed from that obtained in Experiment I and this might be due to a higher concentration of formiminoglutamic acid used in the reaction mixture.

No enhancement in formiminotransferase activity was brought about by addition of vitamin B₁₂ into the reaction systems prepared from the livers of vitamin B₁₂ deficient or normal rats.

Experiment III

In this experiment formiminotransferase activity of the liver was investigated in rats which were given vitamin B₁₂-free diet as long as 70 days. As will be seen from Table IV, no significant decrease in the enzymatic activity was brought about in the liver of these rats in which serum vitamin B₁₂ was markedly reduced, but vitamin B₁₂ content in the liver did not significantly differ from that of

TABLE IV. Experiment III

Group	Nos. of rats	Serum vitamin B ₁₂ (mγ/ml)	Vitamin B ₁₂ content in the liver (mγ/g)	Formiminotransferase activity of the liver*	
				Read at one minute	Read at 3 minutes
II (B ₁₂ -free diet)	27	0.35	179	0.158	0.405
	28	0.52	132	0.205	0.514
	40	0.48	135	0.144	0.390
III (Control)	9	1.33	224	0.148	0.400
	21	1.41	184	0.160	0.425
	22	1.14	253	0.177	0.459

* Liver homogenates were so prepared as to be of protein level of 10 mg/ml. Formiminoglutamic acid in the reaction mixture was 1 micromole per tube.

the control.

CONCLUSION

In summarizing the results obtained from Experiments I-III, the following points should be noticed:

- 1) Vitamin B₁₂ content in serum was markedly reduced in rats which were given vitamin B₁₂-free diet for 30 days or more.
- 2) Vitamin B₁₂ content in the liver was not so markedly reduced as that of serum even when rats were given vitamin B₁₂-free diet as long as 70 days.
- 3) A marked decrease in vitamin B₁₂ content of the liver was brought about in rats which were given vitamin B₁₂-free diet for more than 30 days consecutive to the operation for self-filling diverticulum of the small intestine.
- 4) Formiminotransferase activity of the liver did not decrease in rats, when vitamin B₁₂ was markedly decreased in serum but not in the liver.
- 5) Formiminotransferase activity of the liver was only slightly decreased in certain cases of rats when vitamin B₁₂ was markedly decreased in the liver.
- 6) An addition of vitamin B₁₂ to the assay media did not bring about any enhancement of formiminotransferase activity of the livers from normal or vitamin B₁₂ deficient rats.

Basing upon the results above mentioned we were not in favor of the opinion that vitamin B₁₂ deficiency interfered definitely with the activity of formiminotransferase in the liver of rats.

Acknowledgment

The authors are grateful to Dr. I. Ohara, Surgical Clinic, Tohoku University, for the operation for self-filling diverticulum of the small intestine of rats. This work was supported partly by a grant in Aid for Developmental Scientific Research of Ministry of Education.

References

- 1) Arakawa, Ts., Ohara, K., Kudo, Z., Tada, K., Hayashi, T. & Mizuno, T., *Tohoku J. exp. Med.*, 1963, **80**, 370.
 - 2) Ito, Y., Chiba, R. & Sato, M., unpublished.
 - 3) Knowles, J.O. & Prankard, T.A.J., *Clin. Sci.*, 1962, **22**, 237.
 - 4) Strauss, E.W., Donaldson, R.M.Jr. & Gardner, F.H., *Lancet*, 1961, **11**, 736.
 - 5) Vitamin B Committee of Japanese Society of Vitaminology, *Vitamins (Jap.)*, 1960, **19**, 438.
 - 6) Tabor, H. & Wyngarden, L., *J. biol. Chem.*, 1959, **234**, 1830.
-