Folic Acid Clearance and Its Relation to Initial Levels of Serum Folic Acid Activity in Children

By

Keiichi Hirata and Tsuneo Arakawa

From the Department of Pediatrics, Faculty of Medicine, Tohoku University, Sendai; Director: Prof. Ts. Arakawa

(Received for publication, August 19, 1965)

Folic acid clearance curves, using both *Lactobacillus casei* and *Streptococcus faecalis*, following an intravenous injection of pteroylglutamic acid (PGA) (15 γ per kg of bodyweight) was examined upon 29 children with various diseases. The result revealed that there was a close relation between the initial serum folate activity and the serum folate 240 minutes after the intravenous dose of PGA, and that there were 4 cases with abnormally high levels of serum folate before and after the intravenous injection of PGA.

The purpose of this investigation is to reinvestigate such a result obtained by Hogan *et al.*¹ that there was a correlation between baseline levels of serum folate and folic acid clearance curves. In addition to this, another aim of the present investigation is to confirm an existence of cases with abnormally high levels of serum folate⁸ by using the folic acid clearance test. This is the reason why Herbert and Zalusky² had described that serum folate values, using *Lactobacillus casei*, of more than 25 m γ /ml had never been found in normal subjects unless they were ingesting any vitamin preparations containing folic acid. It had been also reported by these authors² that abnormally high levels of serum folate were found in some cases with vitamin B₁₂ deficiency.

METHODS AND MATERIALS

The children subjected to this investigation were patients admitted into our Hospital.

They were divided into 3 groups according to initial levels of serum folic acid activity for *L. casei*.²

Group I included 18 children (Nos. 1-18) with normal serum folate (6.1-24 m γ /ml); Group II included 7 children (Nos. 19-25) with low serum folate (below

平田慶肇, 荒川雅男

This work was supported in part by a grant of National Institute of Child Health and Human Development, U.S.A., I-ROI-HD-01505-01.

6.0 m γ /ml); and Group III included 4 children with abnormally high levels of serum folate (more than 25 m γ /ml).

The subjects were given 15 γ of pteroylglutamic acid (PGA) per kg of body-weight by rapid intravenous injection. Blood samples were obtained immediately before and at 60, 120, 240 minutes and 24 hours after the injection.

Estimation of the serum folic acid activity was carried out according to Herbert's method,³ with a slight modification by Iwai,¹⁰ by microbiological assay with *Lactobacillus casei* ATCC 7469 and *Streptococcus faecalis* R ATCC 8043.

TABLE I. Folic Acid Clearance Test in Children

No. of case	Name, sex and age	Lapse of time after PGA load Test organisms	Serum folic	
			Before	30 min.
1	S.R. ♂ 10 y.	<i>L. casei</i>	14	34.2
		<i>St. faecalis</i>	1.0	4.4
2	O.K. ♀ 8 y.	<i>L. casei</i>	14	30
		<i>St. faecalis</i>	1.7	4.2
3	O.M. ♂ 6 y.	<i>L. casei</i>	8.1	24
		<i>St. faecalis</i>	<1	1.8
4	O.N. ♂ 9 y.	<i>L. casei</i>	9	24
		<i>St. faecalis</i>	1.1	1.8
5	N.M. ♀ 11 y.	<i>L. casei</i>	11.2	
		<i>St. faecalis</i>	<1	
6	I. I. ♂ 3 y.	<i>L. casei</i>	6	
		<i>St. faecalis</i>	<1	
7	M.M. ♂ 3 y.	<i>L. casei</i>	13.2	
		<i>St. faecalis</i>	<1	
8	S.H. ♀ 14 y.	<i>L. casei</i>	12	
		<i>St. faecalis</i>	<1	
9	S.Y. ♀ 12 y.	<i>L. casei</i>	16.0	
		<i>St. faecalis</i>	<1	
10	K.E. ♀ 11 y.	<i>L. casei</i>	10	
		<i>St. faecalis</i>	1	
11	K.C. ♀ 9 y.	<i>L. casei</i>	9.0	
		<i>St. faecalis</i>	1	
12	T.Y. ♀ 13 y.	<i>L. casei</i>	12	
		<i>St. faecalis</i>	1	
13	A.Y. ♀ 10 y.	<i>L. casei</i>	12	
		<i>St. faecalis</i>	<1	
14	H.T. ♂ 0 y. 10 m.	<i>L. casei</i>	20	
		<i>St. faecalis</i>	<1	
15	S.H. ♂ 9 y.	<i>L. casei</i>	5.8	24
		<i>St. faecalis</i>	1.2	6.2
16	S.T. ♂ 9 y.	<i>L. casei</i>	14	32
		<i>St. faecalis</i>	<1	1
17	S.R. ♀ 5 y.	<i>L. casei</i>	12	30
		<i>St. faecalis</i>	<1	8
18	K.S. ♂ 0 y. 4 m.	<i>L. casei</i>	8.2	
		<i>St. faecalis</i>	<1	

Serum vitamin B₁₂ was estimated by using *Lactobacillus leichmannii* ATCC 4797.⁴

Urinary formiminoglutamic acid (FIGLU) was determined with Tabor and Wyngarden's method.⁵ After an ingestion of L-histidine (0.25 g/kg of bodyweight), urine was collected for 8 hours in a bottle containing 2 ml of concentrated hydrochloric acid.

RESULTS AND DISCUSSION

Group I (children with normal values of initial serum folate for *L. casei*): —
with Normal Baseline Serum Folate (Group I)

acid activity (mγ/ml)				Clinical diagnosis
60 min.	120 min.	240 min.	24 hrs.	
26.4	24	22	14.8	Nephrotic syndrome
1.6	1.3	1.2	1.0	
25	18	17	16.4	Rheumatoid arthritis
3.4	2.9	2	1.9	
19	19	12	9	Bronchial asthma
1.3	1.1	1.1	<1	
19	14	11	9.6	Intracranial bleeding
1.3	1.3	1.3	1.0	
28	24	24		
1.3	1.3	1.3		
12	10	8.2	8.2	Healthy
1.7	1.0	<1	<1	
28	18	17	15	"
4.4	1	<1	<1	
27		18.4		Idiopathic hypoparathyroidism
3.0		1.0		
32.4		21		Congenital nonspherocytic anemia
2.0		1.4		Aplastic anemia
30		18.4		
1.4		1.4		
26		12		Rheumatoid arthritis
3.4		1.4		
30		18		Anemia
3.4		1.4		
38		20		Mental retardation
6		1.7		
32		20		Mental and physical retardation
3.5		<1		tion
		10		Acute hemiplegia
		2		
20		19		Wilson's disease
<1		<1		
20		15		" "
5		4		(sister of Case No. 16)
19		8		Iron deficiency anemia
<1		<1		

The folic acid clearance curves showed that folic acid activity for both *L. casei* and *St. faecalis* returned to the initial levels 24 hours after the intravenous dose (cf. Fig. 1 and Table I).

Group II (children with low values of initial serum folate for *L. casei*): — As were shown in Fig. 2 and Table II, folic acid clearance curves following the intravenous test dose showed lower levels in Group II as compared with those of Group I.

Case No. 19 was a 10-year-old girl, who had been given cow's milk only by gavage for 5 years because she was being comatous since she had suffered from tuberculous meningitis at the age of 5 years. The low value of her initial serum folate was considered to be due to dietary deficiency in folic acid.

Case No. 20 was a 6-year-old girl, who had had frequent attacks of cyclic vomiting and complained of severe anorexia of several years' duration. The low serum folate levels of this patient were thought to be ascribed to an insufficient intake of folic acid.

Case No. 21 was a 11-year-old boy, who had been suffering from pure red cell anemia complicated with hemochromatosis. In this patient folic acid deficiency was considered⁶ to be brought about by defective storage of folic acid coenzyme in the liver due to hemochromatosis.

Case No. 22 was a 3-year-old boy with phenylketonuria, and had been given a low phenylalanine diet — "Lofemilk", Yukijirushi Milk Co. Tokyo, Japan. It was confirmed by us that low content in folic acid of this preparation was respon-

Fig. 1. Folic acid clearance curves of Group I.

Fig. 2. Folic acid clearance curves of Group II.

Fig. 3. Folic acid clearance curves of Group III.

TABLE II. Folic Acid Clearance Test in Children

No. of case	Name, sex and age	Lapse of time after PGA load Test organisms	Serum folic acid activity			
			Before	30 min.	60 min.	120 min.
19	A.K. ♀ 10 y.	L. casei St. faecalis	2.6 <1		10 <1	9 <1
20	S.H. ♀ 6 y.	L. casei St. faecalis	2.7 <1	8.4	9.2 2.4	8.4 1.0
21	Y.H. ♂ 11 y.	L. casei St. faecalis	1.6 0		3.7 1.6	3.4 1.6
22	W.Y. ♂ 3 y.	L. casei St. faecalis	1.6 0	3.4 1	3 0.5	2.8 0
23	K.M. ♂ 3 y.	L. casei St. faecalis	2.2 0		7.0 <1	
24	Y.M. ♂ 11 y.	L. casei St. faecalis	5.2 <1	26	16 4.3	
25	S.T. ♂ 2 y.	L. casei St. faecalis	2.5 <1		7.8 <1	

TABLE III. Folic Acid Clearance Test in Children

No. of case	Name, sex and age	Lapse of time after PGA load Test organisms	Serum folic acid activity (mγ/ml)					
			Before	30 min.	60 min.	120 min.	240 min.	24 hrs.
26	S.K. ♂ 1 y. 1 m.	L. casei St. faecalis	38 1.4		53 11	46 10	42 4	42 2.6
27	C.K. ♀ 3 y. 8 m.	L. casei St. faecalis	25 1		30 8	30 5.8	30	30
28	K.C. ♂ 0 y. 11 m.	L. casei St. faecalis	160 1.0		300 18		280 7.6	
29	O.S. ♀ 0 y. 7 m.	L. casei St. faecalis	120 3.8		220 20		132 10	

with Low Baseline Serum Folate (Group II)

(mγ/ml)		Clinical diagnosis and notes	Urinary FIGLU following L-histidine load (0.25 g/kg)
240 min.	24 hrs.		
6 <1	4 <1	Loss of consciousness She has been fed on cow's milk only by gavage for 5 years since she had suffered from tuberculous meningitis	0.05 μmoles/ml
3.4 <1	2.3 <1	Anorexia Frequent attacks with cyclic vomiting	0.02 μmoles/ml (5.6 μmoles/8 hrs. urine) 0.18 μmoles/ml
3.2 1.2	2.1	Pure red cell anemia with heochromatosis Phenylketonuria He has been fed on a low-phenylalanine diet ('Lofe-milk') for 6 months	0.36 μmoles/ml (203 μmoles/8 hrs. urine) 0.31 μmoles/ml (88 μmoles/8 hrs. urine)
6.4 <1		Acute leukemia 6MP was administered in an amount of 20 mg per day for 21 days	0.82 μmoles/ml, 28/III/1965
6.2 <1		Acute leukemia 6MP was administered in an amount of 30 mg per day for 7 days	0.10 μmoles/ml, 20/IV/1965
4.8 <1		Nephrotic syndrome 6MP was administered in an amount of 25 mg per day for 42 days	0.16 μmoles/ml, 28/III/1965

with Abnormally High Baseline Folate (Group III)

Clinical diagnosis and notes	Urinary FIGLU following L-histidine (0.25 g/kg) load (mole/ml)	Vitamin B ₁₂ in serum (mγ/ml)
Mental retardation	0.37 (Nov. 1964) 0.1 (Dec. ")	0.98 (Nov. 1964) 1.06 (Dec. ") 1.11 (May 1965) 1.37 (June ")
Mental retardation (Formiminotransferase deficiency)	0 (Nov. ")	0.48 0.53 (Apr. ")
Congenital ichthyosis, spastic diplegia, oligophrenia (Sjögren's disease?)	0.15 (June 1965)	1.28 (May ") 1.44 (June ")
Mental retardation	0.03 (June ")	0.75 (June ")

sible for the development of folic acid deficiency of this patient.⁷

Each of three patients (Nos. 23–25) had been given 6MP for a certain period prior to the bioassay for serum folate. The 6MP administration and its influence upon folic acid metabolism are under way of clinical and animal experimentations.

Group III included 4 children with abnormally high values of the initial serum folate. Among them, No. 27 was the patient with 'hyperfolic-acidemia with formiminoglutamic aciduria following histidine loading' reported by Arakawa *et al.*⁸ The other 3 patients were also complaining of mental retardation and showed hyperfolic-acidemia of unknown origin (cf. Table III and Fig. 3).

The folic acid clearance curves in Group III revealed that serum folic acid levels, estimated by *Lactobacillus casei* and *Streptococcus faecalis* 240 minutes after the intravenous dose of PGA, remained abnormally high as compared with those in Group I (cf. Figs. 1 and 3).

Case No. 27 (C.K.) was a 3-year-8-month-old girl, who had been diagnosed as hyperfolic-acidemia with formiminoglutamic aciduria following histidine loading when she was at the age of 8 months.⁸ The folic acid clearance test was carried out when she was 3 years and 8 months old, at this time the abnormally large amount of FIGLU following histidine loading was no more elicited, but

Fig. 4. Correlation between initial serum *L. casei* folate levels and the serum folate 240 minutes after an intravenous injection of PGA (15 γ /kg) in Groups I and II.

Fig. 5. Correlation between initial serum *L. casei* folate levels and the serum folate 240 minutes after an intravenous injection of PGA (15 γ /kg) in Group III.

serum folic acid activity remained relatively high.

Case No. 26 was a 1-year-1-month-old boy with mental retardation. The FIGLU test was positive in Nov. 1964, but became negative soon later (Dec. 1964).

Case No. 28 was a 11-month-old boy complaining of oligophrenia, spastic diplegia and congenital ichthyosis, so clinical diagnosis of Sjörge's syndrome⁹ was made. The urinary FIGLU following histidine loading was found to be 0.15 μ mole/ml, being considered to be slightly increased. Serum vitamin B₁₂ was found to be considerably elevated, being 1.25 m γ /ml—1.44 m γ /ml (cf. Table III).

Case No. 29 was a 7-month-old girl complaining of mental retardation. The urinary FIGLU following histidine loading was found to be within normal range, (0.03 μ mole/ml, June 1965). Serum vitamin B₁₂ was found to be normal (0.75 m γ /ml, June 1965).

These 4 children with hyperfolic-acidemia, included into Group III, were not given any vitamin preparations containing folic acid, and did not show low values of serum vitamin B₁₂.

As was shown in Figs. 4 and 5, it was found that there was a close correlation between the initial serum folate (*L. casei*) and the serum folate 240 minutes after the intravenous injection of PGA (15 γ /kg) in Groups I, II and III.

References

- 1) Hogan, J.A., Maniatis, A. & Moloney, W.C. *Blood*, 1964, **24**, 187.
- 2) Herbert, V. & Zalusky, R. *J. clin. Invest.*, 1962, **41**, 1263.
- 3) Herbert, V. *ibid.*, 1961, **40**, 81.
- 4) Vitamin B Committee of Japanese Society of Vitaminology, *Vitamins* (Jap.), 1960, **19**, 438.
- 5) Tabor, H. & Wyngarden, L. *J. biol. Chem.*, 1959, **234**, 1830.
- 6) Arakawa, Ts., Ohara, K., Kakizaki, R., Takahashi, Y., Hirata, K., Fujii, M.,

- Shibuya, T., Morikawa, T., Chiba, F. & Chiba, R. *Tohoku J. exp. Med.*, 1965, **86**, 301.
- 7) Arakawa, Ts., Hirata, K., Ohara, K., Fujii, M., Takahashi, Y. & Kato, H., *ibid.*, 1965, **80**, 262.
- 8) Arakawa, Ts., Ohara, K., Kudo, Z., Tada, K., Hayashi, T. & Mizuno, T. *ibid.* 1963, **80**, 370.
- 9) Sjörgren, T. & Larsson, T. *Acta psychiat scand.*, 1957, Suppl. **113-116**, 1.
- 10) Iwai, K. *Annual Report of the Japanese Vitamin B Committee*, 1963, p. 76.
-