Effect of Diphenylhydantoin Therapy on Folate Metabolism of Mentally Retarded Epileptics

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ARAKAWA, Ts., YOSHIDA, T., HONDA, Y., NARISAWA, K., HIRONO, H., HAYASHI, T., TSUCHIDA, S., CHIDA, S. and KUWABARA, A. *Effect of Diphenylhydantoin Therapy on Folate Metabolism of Mentally Retarded Epileptics*. Tohoku J. exp. Med., 1973, 110 (1), 59-68 — Either a rise or fall in serum folate levels was observed in mentally retarded epileptics with diphenylhydantoin therapy. Urinary excretion of FIGLU after an oral histidine load was decreased significantly in patients with the anticonvulsant therapy. Eleven cases out of 37 cases with diphenylhydantoin administration showed a positive ferric chloride reaction of urine collected after an oral histidine load. The positive ferric chloride reaction was proved to be due to the presence of imidazole pyruvic acid, suggesting a defective activity of the hepatic histidase of epileptics with the drug therapy. It was suggested that the decreased urinary FIGLU during the drug therapy might be partly resulted from a defective activity of the hepatic histidase which was caused by diphenylhydantoin therapy. Serum vitamin B₁₂ levels, MCV and an incidence of hypersegmentation of neutrophil nuclei showed no difference between patients with diphenylhydantoin therapy and those without it. ——— folic acid; histidase; FIGLU; diphenylhydantoin

The present study was undertaken to investigate an effect, if any, of diphenylhydantoin administration upon serum folate and vitamin B₁₂ levels, MCV of erythrocytes, lobe average of neutrophil nuclei, formiminoglutamic acid (FIGLU) and FeCl₃ tests of urine specimens after an oral histidine load in mentally retarded patients.

MATERIALS and METHODS

Seventy-four mentally retarded patients with or without convulsions, aged between 6 and 28 years, staying at the Nishitaga National Hospital, were subjected to this study.

They were divided into two groups (Groups I and II). Group I consisted of 37 cases (Nos. 1-37) who received no anticonvulsants. Group II consisted of 37 cases (Nos. 38-74) who were treated with diphenylhydantoin plus phenobarbital for their seizures for a period ranging from 6 months to 17 years (cf. Tables 1 and 2).

Blood samples were taken in the morning after a night's fast, and assayed for vitamin B₁₂ and folate (*L. casei*) levels in serum by Lau *et al.*'s method (1965) and a modification of Herbert's method (1961), respectively.

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Formiminoglutamic acid (FIGLU) was estimated by Tabor and Wyngarden's method (1959) on 8-hour-urine specimens after an oral administration of L-histidine monohydrochloride, 0.33 g per kg body weight.

By using the same urine specimens the ferric chloride reaction was tested.

Serum histidine levels were determined using an automatic amino acid analyzer before and at the end of 4 hours after the histidine load.

The lobe average of neutrophil nuclei of the peripheral blood was determined according to Herbert's description (1965).

TABLE 1. Serum vitamin B_{12} and folate levels, MCV, lobe retarded patients without

No. of case	Name	Age (y.)	Sex	Clinical diagnosis and remarks	Serum B_{12} (mγ/ml)
1	T.K.	6	♂	C.P.*	0.52
2	K.K.	6	♂	C.P. Premature	0.98
3	K.T.	8	♂	C.P. Asphyxia	0.58
4	O.S.	8	♂	Down's syndrome	0.45
5	S.R.	6	♂	C.P.	0.98
6	K.K.	10	♂	C.P. Cat's cry syndrome	0.48
7	S.S.	10	♀	C.P. Premature	0.37
8	N.Z.	10	♂	C.P. Sturge-Weber's d.	0.46
9	N.H.	10	♀	C.P. Hydrocephalus	0.23
10	S.B.	11	♀	C.P.	0.19
11	O.M.	12	♀	Hyperalaninemia	0.52
12	M.C.	13	♀	C.P.	0.31
13	S.M.	13	♀	C.P. Encephalopathy at 6 m.	0.35
14	A.U.	14	♀	C.P. Asphyxia	0.23
15	I.M.	14	♀	C.P. Encephalopathy at 1 y.	0.42
16	O.T.	15	♀	Down's syndrome	0.27
17	S.C.	14	♀	Down's syndrome	0.50
18	O.T.	14	♀	C.P.	0.35
19	S.A.	16	♀	C.P.	0.47
20	I.K.	16	♀	C.P. Measles encephalitis at 3 y.	0.40
21	O.S.	16	♂	C.P.	0.35
22	C.I.	17	♂	C.P. Asphyxia	0.28
23	I.M.	18	♀	C.P. Asphyxia	0.20
24	S.K.	18	♂	C.P. Anophthalmos	0.38
25	A.S.	18	♂	Down's syndrome	0.58
26	W.M.	18	♀	B_6 dependent xanthurenic aciduria	0.54
27	U.K.	19	♂	C.P. Kernicterus	0.40
28	N.K.	20	♀	C.P. Asphyxia	0.34
29	A.M.	20	♀	C.P. Measles encephalopathy at 3y.	0.31
30	K.K.	20	♀	C.P.	0.14
31	H.K.	21	♀	C.P.	0.50
32	K.A.	21	♀	C.P.	0.34
33	I.K.	17	♀	Congenital syphilis	0.21
34	D.S.	22	♀	Histidinemia	0.28
35	K.K.	23	♀	C.P.	0.42
36	K.K.	23	♀	C.P. Encephalopathy at 3 y.	0.87
37	I.K.	18	♂	C.P. Asphyxia	0.34
Average					0.43±0.05

* C.P.=cerebral palsy

RESULTS AND DISCUSSION

(1) *Ferric chloride reaction of urine specimens after an oral histidine load in patients with or without diphenylhydantoin therapy:* All the 74 patients were tested for the ferric chloride reaction of urine specimens collected for 8 hours after an oral dose of L-histidine monohydrochloride, 0.33 g per kg. Only 11 cases gave the positive reaction, of which 10 cases (Nos. 42, 46, 49, 50, 57, 59, 61, 62, 64 and 67) were belonged to Group II and the remaining one (No. 34, histidinemia) to

average of neutrophils, urinary FIGLU and FeCl₃ test of mentally anticonvulsant therapy (Group 1)

Serum folate (m γ /ml)	Lobe average of neutrophils	MCV (μ^3)	FIGLU after oral dose of histidine (μ moles per 8 hr. urine)	FeCl ₃ test
11.4	2.49	106	18.2	—
10.0	3.22	94	17.8	—
50.8	2.01	104	43.2	—
10.5	1.66	99	18.9	—
9.1	2.57	93	38.1	—
35.0		100	7.2	—
66.0		77	28.0	—
9.0	2.17	87	33.3	—
13.3	2.40	93	48.0	—
17.2	2.02	93	45.6	—
7.8	2.23	95	35.8	—
12.8	1.98	81	13.6	—
9.7	1.88	120	19.8	—
10.6	2.22	98	26.8	—
5.6	2.38	86	34.2	—
18.2	1.68	103	6.6	—
10.0	2.01	107	33.4	—
8.7	1.66	84	37.1	—
6.1	2.03	99		—
9.3	2.27	96	15.6	—
15.6	2.02	77	30.9	—
6.2	2.16	95	32.2	—
10.1	2.26	78	9.1	—
9.5	2.04	93		—
12.1	1.99	118	4.9	—
13.0	1.83	102	33.3	—
7.7	2.57	87	10.5	—
5.8	1.93	87	37.7	—
10.2	1.52	92	8.8	—
6.6	2.43	91	62.7	—
6.0	2.11	75	14.0	—
5.1	2.32	106	31.1	—
5.6	1.88	87	61.6	—
4.7	2.25	100	32.9	⊕
4.3	2.01	99	40.3	—
2.6	2.48	100	99.0	—
9.8	2.49	101	11.7	—
12.9 $\pm$ 4.3	2.14 $\pm$ 0.18	94.6 $\pm$ 3.7	27.2 $\pm$ 7.5	

TABLE 2. Serum vitamin B_{12} and folate levels, retarded patients with

No. of case	Name	Age (y.)	Sex	Clinical diagnosis and remarks	Serum B_{12} (mg/ml)
38	F.C.	13		C.P.* Asphyxia	0.65
39	S.H.	7		C.P.	0.50
40	Y.M.	7		C.P. Kernicterus	0.20
41	C.Y.	7		C.P.	0.46
42	O.K.	8		C.P. Asphyxia	0.41
43	K.M.	8		C.P. Premature	0.63
44	Y.T.	8		B_6 dependent homocystinuria	0.43
45	Y.T.	9		C.P. Premature	0.52
46	O.E.	9		C.P. Premature	0.67
47	Y.H.	9		C.P. Asphyxia	0.33
48	S.U.	10		Phenylketonuria	0.49
49	O.S.	10		C.P. Kernicterus	0.34
50	O.J.	10		C.P. Asphyxia	0.33
51	Y.K.	10		C.P.	0.22
52	U.K.	11		C.P. Asphyxia	0.53
53	S.N.	11		Tuberous sclerosis	0.24
54	A.E.	12		C.P.	0.20
55	C.T.	12		C.P. Encephalopathy at 4 y.	0.44
56	S.Y.	12		C.P.	0.32
57	T.B.	13		C.P.	0.52
58	T.K.	14		C.P. Encephalopathy at 3 y.	0.32
59	M.K.	14		C.P.	0.64
60	M.E.	15		C.P.	0.21
61	A.Y.	16		C.P. Premature	0.53
62	O.C.	16		Tuberculous meningitis, urocanic aciduria	0.48
63	O.S.	16		C.P.	0.34
64	O.S.	17		Mental retardation (familial?)	0.42
65	S.J.	17		C.P.	0.22
66	S.H.	18		C.P.	0.44
67	T.M.	18		C.P. Tuberculous meningitis, urocanic aciduria	0.20
68	F.K.	18		C.P. Encephalopathy at 2 y.	0.49
69	K.T.	18		C.P. Encephalopathy at 2 y.	1.07
70	I.M.	19		C.P. Encephalopathy at 4 y.	0.60
71	S.T.	20		C.P. Measles encephalitis at 11m.	0.11
72	S.C.	20		C.P. Encephalopathy at 1 y. 2 m.	0.25
73	K.S.	23		Congenital syphilis	0.72
74	S.E.	23		C.P. Encephalopathy at 3y.	0.57
Average					0.43 ± 0.01

C.P.=cerebral palsy

Group I (cf. Tables 1 and 2).

In each of urine specimens with the positive ferric chloride reaction, a considerable amount of imidazole pyruvic acid was detected by a thin-layer chromatography of Humbel's procedure (1970), but no increase in phenylpyruvic acid being found.

In order to evaluate an activity of the hepatic histidase, serum histidine levels were determined before and at the end of 4 hours after an oral dose of L-histidine monohydrochloride, 0.33 g per kg body weight, on 17 patients with

MCV, lobe average of neutrophils, urinary FIGLU and FeCl₃ test of mentally anticonvulsant therapy (Group II)

Serum folate (mγ/ml)	Lobe average of neutrophils	MCV (μ^3)	FIGLU after oral dose of histidine (μ moles per 8 hr. urine)	FeCl ₃ test	Duration of administration of anticonvulsants
8.6	2.45	104	8.6	—	2 y. 7 m.
52.0	1.65	92	2.4	—	3 y. 7 m.
3.2	1.73	100	24.8	—	5 y.
12.1		103	13.5	—	1 y.
33.0	1.95	93	6.2	⊕	2 y. 4 m.
2.6	1.59	87	0	—	2 y. 4 m.
3.3	1.95	97	8.1	—	8 y.
7.6	2.65	110	4.8	—	4 y.
39.3	2.00	88	0	⊕	9 y.
4.9	2.24	89	9.6	—	2 y.
5.2	2.45	129	19.1	—	4 y.
14.6	2.29	67	7.9	⊕	2 y.
57.9	2.03	96	5.2	⊕	4 y.
5.8	1.99	78	7.2	—	4 y.
8.4	2.64	85	26.8	—	4 y. 5 m.
10.1	2.22	98	6.3	—	2 y. 4 m.
6.3	2.53	92	4.0	—	3 y. 5 m.
35.9		82	1.8	—	6 y.
7.8	1.92	109	16.0	—	4 y.
23.0		83	19.8	⊕	0 y. 6 m.
33.1	1.90	98	15.9	—	4 y.
33.0	2.10	133	11.5	⊕	11 y.
3.4	1.91	98	5.1	—	4 y.
47.6	1.56	93	28.4	⊕	2 y. 2 m.
2.4	1.90	108	2.1	⊕	3 y. 5 m.
5.8	1.99	95	14.8	—	4 y.
4.9	2.29	106	26.0	⊕	0 y. 8 m.
37.6	1.75	101	8.1	—	4 y.
3.7	1.69	100	4.4	—	2 y. 3 m.
41.7	1.84	90	5.5	⊕	4 y.
5.6	2.37	86	0	—	2 y. 5 m.
3.7	1.70	81	7.4	—	2 y. 4 m.
7.0	2.74	122		—	2 y. 3 m.
3.6	1.93	90	10.9	—	1 y. 1 m.
5.5	2.00	82	17.9	—	4 y.
5.1		114	7.0	—	3 y.
3.2	1.60	103	8.0	—	17 y.
15.90±4.90	2.04±0.12	96.8±4.5	10.1±2.6		

diphenylhydantoin administration (Group II), of which 5 cases (Nos. 42, 50, 59, 61 and 64) showed the positive ferric chloride reaction of the urine after the histidine load (the positive group), remaining 12 cases (Nos. 40, 43, 48, 55, 58, 60, 63, 65, 66, 69, 73 and 74) having the negative ferric chloride reaction (the negative group). The results, shown in Table 3, revealed that the average of serum histidine levels of the positive group was slightly higher than that of the negative group before and 4 hours after the histidine load, though the difference being not significant between the two groups in this experimental condition.

From these clinical findings it was suspected that a decreased activity of the hepatic histidase might be brought about in some of patients with diphenylhydantoin therapy. This speculation was confirmed to be true by our results of animal experimentation, in which a marked decrease of the hepatic histidase activity was observed in rats given diphenylhydantoin in a dose of 500 mg per kg per day for a period of 65 days (Arakawa *et al.* 1973).

(2) *Urinary FIGLU after an oral histidine load* (cf. Tables 1, 2 and 4 and Fig. 1): The average of FIGLU in 8 hr urine after an oral histidine load was found to be $27.2 \pm 7.2 \mu$ moles and $10.1 \pm 2.1 \mu$ moles in Groups I and II, respectively,

TABLE 3. *Serum histidine levels before and after an oral histidine load in patients with diphenylhydantoin administration*

No. of case	FeCl ₃ test of urine after histidine loading	Serum histidine (mg/100 ml)	
		Before	After histidine loading
40	—	1.09	6.00
43	—	0.66	13.97
48	—	1.29	14.69
55	—	1.12	27.73
58	—	1.23	13.61
60	—	1.44	18.69
63	—	1.23	12.29
65	—	1.35	20.82
66	—	1.16	9.98
69	—	0.82	25.08
73	—	1.36	24.34
74	—	1.24	28.61
Average	—	1.16 ± 0.04	17.98 ± 1.34
42	+	1.66	13.95
50	+	1.69	20.82
59	+	1.33	29.96
61	+	1.26	16.01
64	+	1.26	14.70
Average	+	1.44 ± 0.29	19.08 ± 9.34

TABLE 4. *Distribution of urinary FIGLU after an oral histidine load in mentally retarded patients with or without anticonvulsant therapy*

Urinary FIGLU (moles per 8 hr)	Group I (without anticonvulsant therapy)		Group II (with anticonvulsant therapy)	
	No. of cases	(%)	No. of cases	(%)
> 30	19	(55.6)	0	(0)
29				
> 10	12	(33.3)	13	(36.1)
9				
> 4	4	(11.1)	23	(63.9)
0				
Total	35	(100)	36	(100)

Fig. 1. Urinary FIGLU after an oral histidine load in Groups I and II.

Fig. 2. Serum folate levels of Groups I and II.

the difference between two groups being statistically significant (cf. Tables 1 and 2).

The finding that urinary FIGLU was significantly decreased in patients with diphenylhydantoin administration may be explained in the following way: In our preceding experiments it was found that a marked decrease in the hepatic histidase activity and a marked decrease in urinary FIGLU after a histidine load were brought about in rats by diphenylhydantoin administration. Less yielding of FIGLU from the histidine ingested due to a decreased hepatic histidase activity may contribute to a decreased excretion of FIGLU after a histidine load in rats with diphenylhydantoin administration (Arakawa *et al.* 1973).

(3) *Effect of diphenylhydantoin administration upon serum folate levels* (cf. Tables 1 and 2): The averages of serum folate were found to be 12.9 ± 4.3 μ g/ml and 15.90 ± 4.90 μ g/ml in Groups I and II, respectively, showing no statistically significant difference in the average between two groups. However, Fig. 2 and Table 5 revealed that cases with serum folate above 30 μ g/ml as well as those with serum folate below 5.9 μ g/ml were increased in number in Group II as compared with those in Group I. The difference in the distribution of these cases was found to be statistically significant. This suggested that diphenylhydantoin administra-

TABLE 5. *Distribution of serum folate values of mentally retarded patients with or without anticonvulsant treatment*

Serum folate (mγ/ml)	Group I (without anticonvulsant therapy)		Group II (with anticonvulsant therapy)	
	No. of cases	(%)	No. of cases	(%)
> 30	3	(8.1)	10	(27.0)
29.9 < 6.0	27	(70.1)	10	(27.0)
5.9 < 0	7	(21.8)	17	(46.07)
Total	37	(100)	37	(100)

tion caused either a rise or fall in serum folate levels of epileptic patients.

A decrease in serum folate levels of patients with anticonvulsant therapy has been well documented (Klipstein 1964; Reynolds *et al.* 1966; Dahlke and Mertens-Roesler 1967; Horwitz *et al.* 1968; Jensen and Olesen 1969; Houben *et al.* 1971). As for the mechanism through which subnormal serum folate levels developed in patients with the anticonvulsant therapy, several possibilities have been described, that is, an increased requirement of tetrahydrofolate for parahydroxylation of diphenylhydantoin (Kudd *et al.* 1964; Baylis *et al.* 1971), an interference with folic acid absorption due to inhibition of intestinal conjugase by diphenylhydantoin (Hoffbrand and Necheles 1968; Rosenberg *et al.* 1968) and so on.

However, there has been no report dealing with cases in which serum folate levels showed a tendency to rise during diphenylhydantoin administration.

(4) *Serum vitamin B₁₂ levels in Groups I and II* (cf. Tables 1 and 2): The average of serum vitamin B₁₂ was found to be 0.43 ± 0.05 mγ/ml and 0.43 ± 0.01 mγ/ml in Groups I and II, respectively, showing no difference between both groups.

No influence of anticonvulsant therapy upon serum vitamin B₁₂ levels of 56 epileptic patients, aged between 17 and 61 years, was reported by Jensen and Olesen in 1969.

Malpas *et al.* (1966) reported, however, a decreased serum vitamin B₁₂ level in epileptic patients with anticonvulsant therapy.

(5) *Mean corpuscular volume (MCV) of erythrocytes and the lobe average of neutrophils in Groups I and II* (cf. Tables 1 and 2): The average of MCV was found to be $94.6 \pm 3.7 \mu^3$ and $96.8 \pm 4.5 \mu^3$ in Groups I and II, respectively, revealing no difference between two groups. Macrocytosis ($MCV > 97 \mu^3$) was found in 16 cases out of 37 cases of Group I, and in 17 cases out of 37 cases of Group II, showing no difference in its incidence between two groups.

Eastham and Jancar (1970) reported that macrocytosis in the absence of anemia was demonstrated in the majority of mentally retarded epileptic patients

who aged between 26 and 76 years and were receiving long-term anticonvulsant therapy. Furthermore, they stated that only patients over 25 years of age had been included into their study, because they found that mean red cell volume in non-anemic mentally retarded patients increased with patients' age from childhood onwards, the greatest rate of the increase occurring during a period between 10 years and 25 years of age (Eastham and Jancar, 1970). That the patients subjected to our study were ranging from 6 to 28 years in age might be a reason why a clear-cut effect of diphenylhydantoin administration was not observed in our study.

The lobe average of neutrophils showed also no difference between both groups, that is, being 2.14 ± 0.18 and 2.04 ± 0.12 in Groups I and II, respectively.

Klipstein (1964) made hematologic investigation upon 60 adult patients with anticonvulsant therapy and found that macrocytosis was present in 23 out of 31 cases with subnormal serum folate levels and in 4 out of 22 cases with normal serum folate, and there was none with hypersegmentation of neutrophils in all the cases examined.

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