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Clinical and experimental studies on folic acid deficiency due to anticonvulsants. I. Clinical and nutritional study on megalobastic anemia due to anticonvulsants

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Clinical and experimental studies on folic acid deficiency due to anticonvulsants. I. Clinical and nutritional study on megalobastic anemia due to anticonvulsants*

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Abstract

Two cases of megaloblastic anemia associated with anticonvulsant drugs were studied. Both cases were inpatients of psychiatric hospitals and had quite inadequate intakes of food. The former had lung tuber. culosis and the second had febril illness before the manifestation of anemia. Multiple examinations including bone marrow smears, serum iron levels, vitamin B12 levels, estimation of urinary formiminoglutamic acid after histidine loading and folic acid tolerance test revealed that this anemia was due to folic acid deficiency. Complete hematological responses were ob3erved with injection of folic acid. Retrospective nutritional study on the second case was carried out. The study revealed that folic acid content of the diet of this hospital was 152 fl.g of free folate and 522 fl.g of total folate. The folic acid mtake of the patient was about 80 /1.g of free folate and 280 l1.g of total folate daily during a month before the manifestation of megaloblatic anemia. Importance of additional factors for the development of megaloblastic anemia in patients receiving anticonvulsants was discussed and it was concluded that most important factor was nutritional deficiency of folic acid.

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CLINICAL AND EXPERIMENTAL STUDIES ON FOLIC ACID DEFICIENCY DUE TO ANTICONVULSANTS I. CLINICAL AND NUTRITIONAL STUDY ON MEGALOBLASTIC ANEMIA DUE TO ANTICONVULSANTS

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Megaloblastic anemia due to anticonvulsant drug therapy was first reported by MANNHEIMER *et al.* (1) in 1952. Many case reports and investigations on the anemia have hitherto been carried out. In Japan, the first case was reported by the author in 1967 (2). Three other cases have been recorded in Japan thereafter (3)(4)(5). The fifth case in Japan was observed by the author recently. Attention has been directed to rare occurrence of the megaloblastic anemia in patients receiving anticonvulsant drug therapy. Additional factors other than the ingestion of anticonvulsant drugs appear to be operative in the development of sufficient folate deficiency to produce a megaloblastic anemia. Inadequate diet was thought to be one of the most important factors for the aggravation of folic acid deficiency in the two cases observed by the author. Therefore, case history of the two cases was briefly described and retrospective nutritional studies were carried out to show that malnutrition plays a role for the manifestation of severe anemia in these cases.

SPECIAL LABORATORY METHODS

Folic acid activity of serum, cerebrospinal fluid (CSF) and urine were assayed by the method described by WATERS and MOLLIN (6) using *Lactobacillus casei* (L. casei ATCC 7469) as the test organism. Difco folic acid casei medium was employed. Normal value in this laboratory was 3.5-20 ng/ml. Serum and CSF vitamin B_{12} (B_{12}) activities were determined by the method of Japan Vitamin Society (7) using *L. leichmannii* (ATCC 7830). Normal value was 150-900 pg/ml.

D-Xylose absorption test was performed by the technic described by BENSON *et al.* (8). Sideroblastogram was drawn by the way advocated by KIMURA (9). Urinary formiminoglutamic acid (FIGLU) secretion after oral histidine loading was estimated according to the technic described by CHANARIN and his associates

(10). Folic acid clearance study was performed by the method of CHANARIN *et al.* (11). Estimation of food folate contents was made by the technic of HERBERT (12). Slight modification was made as follows (13). One or two grams of foods was homogenized with 0.1 M phosphate buffer (pH 6.0) containing 150 mg% of ascorbic acid in glass homogenizer. The homogenate was autoclaved at 121°C for 10 minutes. After centrifugation, a half of the clear supernatant was set aside for estimation of total folate. The another half of the supernatant was diluted with water adequately for assay of free folate. Total folic acid, which implies pteroylpolyglutamates conjugated by three to seven glutamic acids, was estimated with the former half of the supernatant. After incubating them separately for 24 hours at 37°C by adding 3 mg/10 ml of Difco dessicated chicken pancreas, they were autoclaved for 5 minutes at 121°C. Centrifigation was made and clear supernatants were diluted adequately and assayed respectively for free and total folic acid. The way how to calculate folic acid contents of the hospital diet will be shown in the part of special nutritional study of this paper.

CASE REPORTS

Case. 1. A 34-year-old male, who had been admitted in a psychiatric hospital on a diagnosis of psychomotor epilepsy since 1955, had severe anemia in the end of September, 1965. He had not received any anticonvulsant until May 6, 1965, when he was administered 100 mg of diphenylhydantoin and 100 mg of phenobarbital for the first time as a epileptic attack (grand mal type) recurred since April of that year. Hematological examination performed a month after initiation of the anticonvulsant therapy showed red cells 4, 300, 000 per cubic milliliter (cu. mm.), hemoglobin (Hb) 97% and white cells 6, 200 per cu. mm.. His dietary history at the hospital was very capricious and he frequently refused to take hospital diet. In September 1965, he complained of abdominal pain and anorexia. There were no vomiting and diarrhea. On 20th, September, fever of 38.5°C occurred and continued every day. He became pale and general weakness was aggravated. On 28th, September, precise clinical examination was performed. The patient was an asthenic man, who appeared not to be malnourished. The skin was pale but edema, exanthema and petechiae were not detected. The tongue appeared normal. Pulse rate was 72 per minute and blood pressure, 100/56 mmHg. Slight degree of systolic murmur was heared at the apex. Auscultation of the lung revealed moist rales at the right upper lobe. The liver and spleen were palpable 1 cm below the costal margin. The kidneys were also palpable. Neurological examinations did not disclose any abnormalities.

Initial laboratory study revealed Hb of 40%, red cells 1, 770, 000 per cu. mm., reticulocytes 1.2% and a platelet count of 92, 000 per cu. mm.

The count of white cells was 4, 600 per cu. mm. with a differential of 2.5 % of promyelocytes, 1.5% myelocytes, 2.5% metamyelocytes, 12.5% band netrophils, 63.5% segmented neutrophils, 1.5% eosinophils, 1.5% monocytes, 14.5% lymphocytes. Microscopic examinations of peripheral blood smears revealed macrocytosis, anisocytosis, polychromatic erythrocytes, Jolly bodies, Cabot rings in erythrocytes and hypersegmented polymorphonuclear cells. Serum iron level was 163 μg/dl. Gastric juice showed normoacidity. Roentgenograms revealed normal upper gastrointestinal and small bowel series. The chest film showed the presence of lung tuberculosis at the right upper lobe. Tubercle bacilli were negative in the sputum. Sternal marrow aspiration smears revealed a typical megaloblastic pattern. 39.4% of the nucleated cells were megaloblasts and giant metamyelocytes were observed. Findings from the following examinations were all normal; relative to urinalysis, stool (parasite eggs and occult blood), liver function tests, serum cholesterol, serum protein, electrolytes, NPN, PSP, fasting blood sugar, glucose tolerance tests, amylase levels in serum and urine, electrocardiogram and ocular fundi.

As assay of serum folic acid and B_{12} was impossible at that time, diagnosis ex juvantibus was employed (Fig. 1). Since 7, October, injection of 2, 000μg of hydroxocobalamin was made without subsequent reticulocytosis even after one week. All the anticonvulsants were stopped since 12 Oct. Pteroylglutamic acid (15mg) was injected intramuscularly since 14 Oct. Two days after the initiation of folic acid, bone marrow became normo-

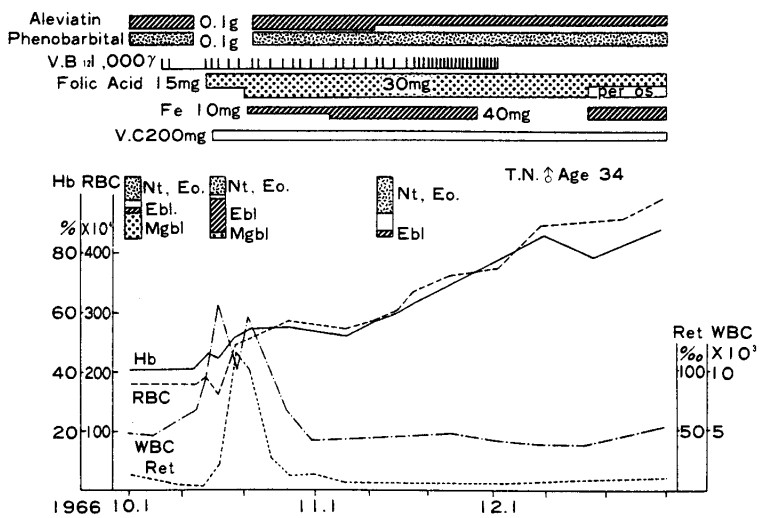

Fig. 1 Blood changes and therapies in Case 1.

blastic. On the fifth day of the treatment, reticulocytes' peak of 11.6% was observed. Appetite improved within three days. Epigastralgia, nausea also disappeared rapidly after administration of folic acid.

Streptomycin, isonicotinic acid, sulphonamide were administered for the lung tuberculosis. On 20 Oct., eighth day after the cessation of anticonvulsants, a severe attack of epilepsy occurred. Anticonvulsants were administered again since that time. No increase in fit frequency was observed since then despite the administration of folic acid. Parenteral iron supplements were employed for the relative iron deficiency by a rapid recovery of the anemia. These procedures gave rise to complete hematological and clinical remission within two months. This case was the first report of megaloblastic anemia due to anticovulsant in Japan.

Case 2. A twenty-year old male, who had been admitted to Fuchu Hospital since January of 1967 as psychomotor epilepsy, was referred to a internist of the hospital for his severe anemia on 14 Oct. 1970. He had been taking 60mg of phenobarbital since February of 1968, then Comital L (containing 50 mg of diphenylhydantoin, 50mg of prominal and 50 mg of phenobarbital in a tablet) two tablets a day since May, 1968. Comital L was increased up to 3 tablets a day since September 1969. The attack of epilepsy had been infrequent but he had shown explosive disposition and autistic proneness. He had been irascible and violent to other patients frequently. His dietary habits had been very fluctuating. He had not eaten vegetables at all. Occasionally he refused to eat anything all day long. In March 1970, hematological examinations revealed red cells 4, 150, 000 per cu. mm., Hb 15.9g/dl, and white cells 4, 400 per cu. mm. In June, slight degree of anemia (red cells 3, 580, 000 per cu. mm.) was detected. No attention was paid to this fact by his doctor at that time. In September, he had fever, cough and sore throat. His appetite was lost markedly. Nausea, vomiting and sore tongue made him not to take hospital diet sufficiently. At the end of September, the nurses noted paleness of his face. In October. 1970, nausea, vomiting, dizziness and general weakness were aggravated. So examinations on the cause of the anemia were asked of the author.

Physical examination showed a well nourished, middle statured man. No edema and petechiae were observed in the skin. No palpable lymph nodes were detected. Conjunctiva was anemic but not icteric. Tongue was not atrophic but had slight hyperemic appearance. Gingiva showed no hypertrophy. Body temperature was 36.7°C. Pulse rate was 76 per minute and blood pressure 120/70 mmHg. Evidence of cardiomegaly was not observed. A grade II systolic cardiac murmur was heard best at the

apex. Lungs were clear. No abnormalities was found in the abdomen and at no time was there any subjective or objective evidence of any neurological abnormalities.

Examination of his blood showed red cells 1,430,000 per cu. mm., Hb 5.3g/dl, packed cell volume 16%, mean corpuscular volume (MCV) 112 cu. μ., mean corpuscular Hb concentration 34%, reticulocytes 0.4%, platelets 180,000 per cu. mm., leucocytes 2,150 per cu. mm. (polymorphs 62%, eosinophils 1%, lymphocytes 37%)

Stained blood film showed macrocytosis, anisocytosis and hypersegmented neutrophils. Erythrocyte sedimentation rate was 45mm/hour. Urinalysis and stool examination were normal. Serum iron level was 500 μg/dl. Serum LDH showed a high level of 1130 unit. Examinations of serum revealed cholesterol 186 mg/dl, bilirubin 0.50 mg/dl, SGOT 19, SGPT 14 Karmen Unit, Alkaline phosphatase 6.9 King-Armstrong units, and serum protein 7.3 g/dl with A/G ratio of 2.09. The chest roengenogram, gastrointestinal series with small bowel study were within normal limits.

Free hydrochloric acid was present in the gastric juice. Bone marrow aspiration was not performed at the time of the first examination. Total of seven units (1,400 ml) of blood transfusion, parentheral iron and B_{12} were given on the following seven consecutive days. To this treatment there were no reticulocyte response. Marrow aspiration was carried out on 24, October. The stained film revealed the presence of 30.8% of megaloblasts. Giant metamyelocytes were also present. Sideroblastogram disclosed a type of pernicious anemia. Serum folic acid level was 0.5 ng/ml. Serum B_{12} level was 2,375 pg/ml. FIGLU excretion was 110.5 mg (normal 0-17 mg). CSF folic acid level was 8 ng/ml (normal 5-50) and CSF B_{12} level was 60.7 pg/ml (normal 0-30). Folic acid clearance test was made by intravenous injection of 15 μg/kg of folic acid. The peak of serum folic acid level obtained after injection of folic acid was lower in the patient than in control. Urinary excretion of folic acid was also reduced in the patient. These results showed that there was folic acid deficiency in the patient (Table 1). The course and therapy are shown in

TABLE 1. FOLIC ACID CLEARANCE STUDY

Time (min.)	Serum Folate Levels after 15μg/kg of Folic Acid (ng/ml)					Urinary Excretion (μg/day)
	5	15	30	60	120	
Patient	50	20	12.5	8.7	7.5	87.5
Control	110	92	58.5	44	40	210

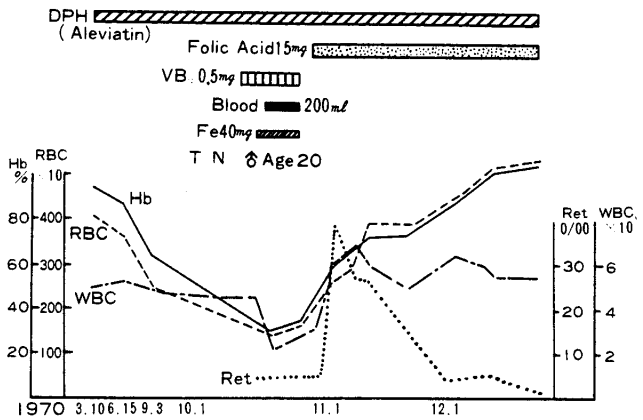

Fig. 2 Blood changes and treatments in Case 2.

Fig. 2. 720 μg of folic acid was injected on 31, Oct. for the folic acid clearance test. Then 15 mg of folic acid was injected twice a week. Reticulocytes reached its peak of 3.4% three days after the injection of folic acid. A week later, bone marrow became normoblastic. A good hematologic response was obtained without discontinuing the anticonvulsants. The administration of folic acid had no effect on fit frequency. The blood picture improved rapidly and on December 4, it showed: red cells, 4, 460, 000 per cu. mm., Hb., 14.6 g/dl, white cells, 6, 100 per cu. mm.

NUTRITIONAL STUDY

The anemia in the second case was severe from July to September, 1970, that is before folic acid therapy. A retrospective nutritional study on this period on the patient was carried out to ascertain the cause of this anemia.

1 *Dietary history*:

Amounts of foods he had taken daily during these period were obtained by the clinical records of that hospital. The rate of food intake of all the given hospital diet is shown in Fig. 3. During the period of August 20 to 31, he had taken all given diet in six of twelve days. In September he took full diet only two days. For fifteen days he took less than a half of the daily diet. His food intake was less than 80% for other eleven days.

2 *Folic acid contents of each food*:

Folic acid contents of various food were assayed by the method described in the chapter of special laboratory methods of this paper. The

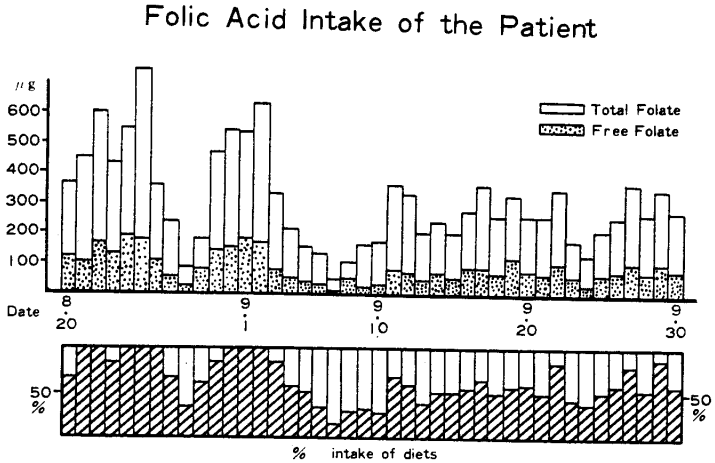

Fig. 3 Intake of diets and folic acid in Case 2.

TABLE 2. FOLIC ACID CONTENTS OF MAIN FOODS.

THE TERM "FREE" INDICATES FOLIC ACID CONTENT OF FOOD ASSAYED BEFORE TREATMENT OF CONJUGASE. "TOTAL" MEANS FOLIC ACID ASSAYED AFTER CONJUGASE. IN PARENTHESIS THE WAY OF COOKING WAS SHOWN.

(μg/g)

	Free	Total		Free	Total
Rice (boiled)	0.024 ~ 0.070	0.200 ~ 0.345	Tuna, sliced	0.040	0.243
			Whale (boiled)	0.008	1.155
Bread	0.035	0.750	Spinach	1.280	2.105
Potatoes (boiled)	0.027	1.692	Lettuce	0.750	1.340
Eggs, white	0.007	0.066	Cabbage	0.483	1.270
yolk	1.300	1.800	Leak, green	0.704	2.006
Beef (steak)	0.105	0.991	Radish	0.450	0.770
Fork (steak)	0.011	0.912	Cucumbers	0.290	1.017
Chicken (boiled)	0.140	0.408	Carrot	0.235	0.456
Lobster (broiled)	0.855	1.710	Onions	0.020	0.062
Mackerel (boiled)	0.166	0.788	Apples	0.040	0.100
horse Mackerel (boiled)	0.144	0.344	Tomatoes	0.500	0.840
Salmon (salted)	0.024	0.440	Bananas	0.350	0.966
Facific Saury (broiled)	0.090	0.410	Mandarin Oranges	0.260	0.294
Octopus (boiled)	0.035	0.180			

results have been reported previously (13). Some of them are quoted in Table 2.

3 *Calculation of the folic acid intake of the patient.*

An example of the way of how to calculate the folic acid intake is shown in Table 3. Materials of each meal are presented at the first column.

TABLE 3. AN EXAMPLE OF THE WAY OF CALCULATION OF FOLIC ACID INTAKE OF THE PATIENT.

Menu		Amount	Unit folate content (μg/g)	Food folate content (μg)	Intake of diet by patient	Folate intake of the patient (μg)
Break fast	rice	250	0.047	11.8	$\frac{7}{10}$	8.3
	soy soup			17	$\frac{5}{10}$	8.5
Lunch	rice	250	0.047	11.8	$\frac{7}{10}$	3.5
Omlet	egg	50	0.47	23		
	onion	40	0.02	0.8		
	carrot	5	0.1	0.5	$\frac{3}{10}$	15.6
	pea	3	0	0		
	minched chicken	20	0.14	2.8		
Cabbage		30	0.48	14.5		
Salted greens						
	cucumber	50	0.2	10		
	carrot	5	0.1	0.5		
Supper	rice	250	0.047	11.8		
	octopus	70	0.082	5.7		
	radish	20	0.45	9.0		
	cucumber	10	0.29	2.9	0	0
	potato	80	0.027	2.2		
	pork	10	0.02	0.2		
	green pepper	10	0.02	0.2		
	Total			124.7		35.9

The second column shows amount of each materials. The third one is folic acid content of the foods. The next column, folic acid contents of each food, is obtained by multiplying the value of the second column by that of the third one. The fifth column is the ratio of his food taking. The final column indicates the amount of folic acid taken by the patient from each materials. According to this table, he took 35.9 μg of free folate of 124.7 μg of given foods on September 6. Calculations of folate intake of the other days were carried out in the same way. Results were presented in Table 4. Mean folic acid supply by diets of this hospital was 152 μg of free folate and 522 μg of total folate. From August 30, mean free folate intake of the patient was 123 μg of 151 μg of given folate of the hospital diet and mean total folate 412 μg of 509 μg, and the patient took about 80 μg of free folic acid and 280 μg of total folic acid during

TABLE 4. FOLIC ACID INTAKE OF THE PATIENT. THE AMOUNTS SHOWN IN PARENTHESES ARE THAT OF FOLIC ACID GIVEN AT THIS HOSPITAL.

Date	Free Folate	Total Folate	Date	Free Folate	Total Folate
20, Aug.	125 (171)	371 (552)	31	154	539
21	119	452	1, Sep.	182	522
22	169	600	2	169	636
23	131 (152)	426 (520)	3	85 (141)	327 (553)
24	193	554	4	54 (112)	215 (430)
25	189	740	5	41 (204)	166 (625)
26	116	373	6	36 (125)	123 (597)
27	64 (102)	254 (282)	7	8 (105)	48 (463)
28	29 (122)	98 (458)	8	51 (165)	108 (504)
29	79 (164)	195 (525)	9	24 (139)	136 (504)
30	142 (168)	471 (539)	10	32 (186)	189 (613)
Mean	123 (151)	412 (509)	Mean	76 (153)	274 (544)
Date	Free Folate	Total Folate	Date	Free Folate	Total Folate
11, Sep.	84 (134)	354 (504)	21, Sep.	70 (140)	270 (540)
12	72 (147)	330 (544)	22	106 (129)	357 (537)
13	50 (172)	210 (566)	23	65 (167)	194 (537)
14	74 (149)	247 (494)	24	32 (198)	133 (585)
15	56 (156)	200 (488)	25	70 (139)	225 (550)
16	97 (180)	271 (523)	26	82 (109)	264 (421)
17	93 (137)	378 (533)	27	117 (150)	382 (484)
18	69 (132)	257 (473)	28	76 (168)	270 (582)
19	123 (165)	329 (531)	29	117 (126)	352 (441)
20	82 (170)	269 (507)	30	82 (178)	279 (515)
Mean	80 (154)	284 (516)	Mean	82 (150)	273 (519)

September, when his anemia might be aggravated.

DISCUSSION

Megaloblastic anemia due to anticonvulsants is very rare. In Japan, only five cases have been reported. In this paper the first and fifth cases are recorded. Anemia was detected in psychiatric hospitals. They had severe megaloblastic anemias during the course of anticonvulsant therapy. Differences from the classic type of pernicious anemia was the presence of free hydrochloric acid in gastric juice and absence of neurological disturbances. The nature of the anemia was clearly by folic acid deficiency. In the first case, therapy by B_{12} was not effective. Administra-

tion of folic acid alone could induce a clinical and hematological response. In the second case various examinations on folic acid metabolism were carried out. Serum folic acid level was extremely low. FIGLU excretion after histidine loading was increased. Folic acid tolerance test revealed low peak serum level of folic acid and less urinary folate excretion. There is no doubt that megaloblastic anemia during anticonvulsant therapy is due to folic acid deficiency. Though the mechanism by which anticonvulsants cause folic acid deficiency is yet unknown (14), evidence suggesting frequent occurrence (as high as 75%) of subclinical folic acid deficiency among patients receiving anticonvulsant drugs was known (15)(16). Many other observations in non-anemic patients receiving anticonvalsants (17) (18) (19) (20) (21) (22) showed a high incidence of macrocytosis and low serum folate levels. These observations suggest that folic acid deficiency caused by anticonvulsants is not so severe in its degree. Some additional factors may be required for the initiation of severe megaloblastic anemia. Two cases presented in this paper were inpatients of psychiatric hospitals. Many other reports (23)(24)(25) noted high incidence of this anemia among mentally defective or psychotic patients. The dietary intake of mentally defective patients is frequently inadequate as was the case in the two patients reported in this paper. Therefore the importance of nutritional factors for the occurrence of megaloblastic anemia due to anticonvulsants was suggested. NEWMAN *et al.* (26) and KIDD *et al.* (25) reported cases in whom malnutrition were observed. FLEXNER *et al.* (27) were the first that performed nutritional studies on the case of megaloblastic anemia due to anticonvulsants. Their studies were composed of taking dietary history, folic acid tolerance tests, estimations of serum vitamin C level, vitamin A level, carotene level and iron level. Absorption test on B_{12}, glucose and triolein were also included. These analysis disclosed poor intake of folic acid, vitamin C and of some of vitamin B complexes. Amounts of folic acid taken by the patient was not studied.

The present study may be the first that investigated the amount of folic acid which the patient had taken before the onset of megaloblastic anemia. The second patient received diphenylhydantoin, phenobarbital and prominal since May, 1968. He was not anemic in March 1970. Anemia was dicovered in October 1970. Folic acid deficiency was rapidly aggravated in these period. The clinical record of this hospital showed that his dietary intake was extremely diminished in August and September of that year. Even the half of the given diets were not taken by the patient in September, that was a month before his anemia was detected. Accordingly folic acid intake from diets was diminished. Mean folate

content of the hospital in these period was calculated as 152 μg of free folate and 522 μg of total folate. Folic acid content of hospital diets in Japan was reported by IWASAKI and SANADA (28). According to their work assay was made in two hospitals. Free folate of 108 μg/day and total folate of 422 μg/day were the mean daily folic acid content of the hospital A. In hospital B in their work, mean free folate per day was 138 μg and total 351 μg. The values obtained in the present study were comparable to these values.

BUTTERWORTH *et al.* (29) showed that American diet contained 157 μg of free folate and 688 μg of total folate per day. CHANARIN *et al.* (30) reported that healthy pregnant women in England took 160 μg of free folate and 676 μg of total folate daily from their diets. These values are essentially in agreement with the values found in the present study. Availability of food folate is different by its form. Monoglutamate form of folate are readily absorbed than polyglutamate. About 80% of monoglutamate form of folate in foods is absorbed (31). Most of the folate in foods is present in the form of polyglutamate. PERRY and CHANARIN (32) described that some 27% of polyglutamate forms of folate was absorbed. Calculated amount of folate by applying the values was about 263 μg in this hospital.

Minimal daily requirement of folic acid has been considered to be 50 μg (33) to 100 μg (34). Therefore, folic acid was adequately supplied in this hospital. As was demonstrated in Table 3 and Fig. 3, folic acid intake of the patient studied in this paper was very poor in September. Mean was about 80 μg of free folate and 280 μg of total folate during this period. These amounts were about half of the given folate. Considering the absorption rate, some 140 μg of folate could be absorbed if inhibition of folate absorption was not present.

At present a hypothesis that anticonvulsants interfere with absorption of folic acid is almost proved (35) (36) (37). Therefore, folic acid absorbed by the patient in the present study was much less. Folic acid deficiency of the patient was aggravated and megaloblastic anemia became manifest during this period. Though coincident occurrence of infection in these patients may play a role for the development of the anemia by increasing folic acid requirement (38) (39) (40), it is concluded that inadequate intake of folic acid from foods is the most important factor for the manifestation of megaloblastic anemia due to anticonvulsants. This fact suggests that megaloblastic anemia will not be seen in patients receiving anticonvulsants if their diet intake is adequate. This conclusion explains well the question why megaloblastic anemia is so rare among patients receiving anticonvul-

sants and why the anemia is relatively frequent in mentally defective men who seem to have peculiar diet habits.

CONCLUSION

Two cases of megaloblastic anemia associated with anticonvulsant drugs were studied. Both cases were inpatients of psychiatric hospitals and had quite inadequate intakes of food. The former had lung tuberculosis and the second had febril illness before the manifestation of anemia. Multiple examinations including bone marrow smears, serum iron levels, vitamin B_{12} levels, estimation of urinary formiminoglutamic acid after histidine loading and folic acid tolerance test revealed that this anemia was due to folic acid deficiency.

Complete hematological responses were observed with injection of folic acid. Retrospective nutritional study on the second case was carried out. The study revealed that folic acid content of the diet of this hospital was 152 μg of free folate and 522 μg of total folate. The folic acid intake of the patient was about 80 μg of free folate and 280 μg of total folate daily during a month before the manifestation of megaloblatic anemia. Importance of additional factors for the development of megaloblastic anemia in patients receiving anticonvulsants was discussed and it was concluded that most important factor was nutritional deficiency of folic acid.

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