Serum Folic-acid and Vitamin-B₁₂ Levels in Anticonvulsant Therapy

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The association of megaloblastic anaemia with anticonvulsant therapy was first reported by Mannheimer, Pakesch, Reimer, and Vetter (1952). Since then some cases of megaloblastic anaemia associated with a variety of anticonvulsants have been reported. The finding of normal levels of serum vitamin B₁₂ and the repair of the anaemia which follows folic-acid therapy suggest that anticonvulsants affect folic-acid metabolism. Further evidence for this has been the demonstration of macrocytosis or low serum folic-acid levels in about half the patients who have been on anticonvulsant drugs (Hawkins and Meynell, 1958; Klipstein, 1964).

The influence of diet on the results of these surveys has not been clear. In order to investigate this, serum folic-acid and vitamin-B₁₂ levels were studied in a group of epileptics and normal control subjects who were on identical and satisfactory diets.

Methods and Materials

The epileptic patients studied were in-patients at Chalfont Colony, Chalfont St. Peter, Buckinghamshire. The great majority of patients were taking phenobarbitone and phenytoin. A few were receiving primidone instead of or in addition to phenytoin, and some were also being treated with Trinuride. There they take their meals in small groups supervised by ward sisters. The nursing staff are able, therefore, to know the dietary habits of each patient. Every patient studied was interviewed in the presence of the sister in charge, and this was particularly helpful in assessing the diet.

A 10% random sample of Chalfont Colony was taken after numbering all the patients and then selecting them by means of random numbers (Hill, 1961). Forty-eight patients were chosen in this way; a further 12 who had been in poor health and who had been noted to have a low white blood cell count were also studied.

Twenty nursing sisters and ward staff were used as control subjects. They were all residents, and their diet was identical to that of the patients and was prepared in the same kitchens.

The levels of haemoglobin, haematocrit, mean corpuscular haemoglobin concentration, and mean corpuscular volume were estimated by the methods of Dacie (1964). The assessment of macrocytosis was done independently by three observers who were unaware of the results of the serum folic-acid assay.

The serum vitamin-B₁₂ levels were determined by microbiological assay, *Lactobacillus leichmannii* being used as test organism (Spray, 1955). By this method the normal range is 150-1,000 µg./ml. (Spray and Wits, 1958); values in the 20 control subjects in this study ranged from 250 to 920 µg./ml. Serum folic acid was assayed with *L. casei* by the method described by Spray (1964), which gave a normal range of 2.1-28 µg./ml. The normal limits in the present group of control subjects were 2.1 to 10 µg./ml.

Results

None of the patients studied was anaemic. Full haematological data on those patients whose serum folic-acid level was 2.1 µg./ml. or less are shown in the Table. Four of

the randomly selected patients showed macrocytosis associated with a low serum folic-acid level. A further 11 had a serum folic-acid level of 2.1 µg./ml. or less (Fig. 1). The mean level was 2.75 µg./ml., compared with 5.7 µg./ml. in the controls. This was significant at the 0.1% level.¹ Two of these patients (Cases 2 and 7 in the Table) showed levels of serum vitamin B₁₂ at the lower end of the normal range; the mean serum vitamin-B₁₂ level for the group was 372 µg./ml., compared with 475 µg./ml. in the controls (Fig. 2). This difference is significant at the 1% level.²

There was no relation between the duration of therapy or the dose of anticonvulsants and the serum folic-acid or serum vitamin-B₁₂ levels.

Of the 12 patients studied because of symptoms, two showed macrocytosis and six showed low levels of serum folic acid. The dietary intake of foods containing folic acid was assessed as very poor in one of these patients (Case 16 in the Table) and impaired in four more. This was in contrast to the excellent dietary state of the randomly selected group.

In-vitro studies of the effect of anticonvulsant drugs showed that phenobarbitone and phenytoin sodium had no effect on the serum folic-acid assay when added to the medium in amounts comparable to those likely to occur in the extracts of the patients' sera.

Discussion

It now seems very likely that the macrocytosis and megaloblastic anaemia which may develop in patients on anticonvulsant therapy are due to an abnormality of folic-acid metabolism. Low levels of serum folic acid have been found by Kohn, Mollin, and Rosenbach (1961) and by Druskin, Wallen, and Bonagura (1962) in patients with megaloblastic anaemia due to anticonvulsants. Chanarin, Mollin, and Anderson (1958) showed a rapid clearance of folic acid from some of these cases, suggesting a deficiency of folic acid in the tissues. The low folic-acid levels observed are not due to an inhibitory effect on the assay; this repeated observation has been confirmed in the present study.

Macrocytosis or low serum folic-acid levels have been noted in patients on anticonvulsants for epilepsy secondary to focal brain damage (Hawkins and Meynell, 1958). Four of our five cases of post-traumatic epilepsy showed low folic-acid levels (Fig. 1). It seems unlikely, therefore, that there is an inherent defect in folic-acid metabolism associated with cryptogenic epilepsy.

There is no evidence of impaired folic-acid absorption in epileptics. It has been shown that the urinary excretion of folic acid after a test dose (Girdwood, 1953) is normal in these cases (Girdwood, 1956). The haematological recovery that occurs without the institution of any other therapy when anticonvulsant drugs are stopped and the recurrence on renewal

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¹ A log transformation was done to express the variances of the two groups of folic-acid measurements. The value for *t* was 6.634, *n*=68, *P*<0.001.

² Testing the differences between the means, *t*=2.74, *n*=68, *P*<0.01.

of these drugs suggest that anticonvulsants affect the metabolic pathway of folic acid (Christenson, Ullmann, and Roseman, 1957). This direct action is supported by the haematological surveys of Hawkins and Meynell (1958) and Klipstein (1964), and by the results of the present study.

The previous studies showed an incidence of 45% of patients with abnormalities suggesting derangement of folic-acid metabolism. In our randomly selected group on com-

parable doses of the same anticonvulsants the incidence of macrocytosis was 11% and low serum folic-acid levels 37%. The diminished incidence of macrocytosis is striking. In the absence of any other difference between the present survey and those previously carried out it would seem that a satisfactory diet may exercise some measure of protection. Klipstein's survey was, for example, on patients treated on an out-patient basis. Presumably a number of these patients

might find difficulty in employment and might gravitate to a lower social scale because of their complaint. Further evidence for this view comes from our second group, who had been in poor health and whose diet had been unsatisfactory for a number of reasons. In these subjects the incidence of macrocytosis was twice that seen in the randomly selected group, and low serum folic-acid levels were noted in 50%.

Low levels of serum vitamin B₁₂ have been noted in patients on anti-convulsants, and reticulo-cyte responses to injected vitamin B₁₂ have occurred (Badenoch, 1954; Kidd

FIG. 1

FIG. 2

Haematological Findings on 21 Patients whose Serum Folic Acid was 2.1 µg./ml. or Less

Case No.	Age and Sex	Anticonvulsant	Dose (mg./day)	Duration (years)	Hb (g./100 ml.)	Hct (%)	M.C.H.C. (%)	M.C.V. (c.µ)	Macrocytosis + or -	Serum B ₁₂ (µg./ml.)	Serum Folate (mµg./ml.)
Patients Randomly Selected											
1	36 M	Phenytoin sodium Phenobarbitone	200 100	6	16.6	48	35	90	-	250	1.8
2*	20 M	Phenytoin sodium Phenobarbitone	300 150	14	14.8	46	32	98	+	160	1.6
3	52 M	Phenytoin sodium Phenobarbitone	200 130	14	15.0	45	33	89	-	320	2.0
4*	45 M	Phenytoin sodium Phenobarbitone	300 150	5+	14.6	46	32	100	+	320	1.0
5	73 M	Phenytoin sodium Phenobarbitone	200 160	15	14.8	47	31	105	+	520	1.1
6	48 M	Phenytoin sodium Phenobarbitone	350 150	13	14.5	43	34	94	-	290	1.7
7	40 M	Phenytoin sodium Phenobarbitone	300 150	12	14.8	43	33	93	+	160	1.3
8	50 M	Phenytoin sodium Phenobarbitone	300 150	14	15.8	47	34	92	-	370	2.0
9	60 M	Phenytoin sodium Phenobarbitone	200 50	16	14.6	48	30	96	-	800	1.3
10*	54 F	Phenytoin sodium† Phenobarbitone	200 100	15	14.5	44	33	99	-	260	1.6
11	50 F	Phenytoin sodium Primidone	200 750	20	14.1	44	32	98	-	290	2.0
12	40 F	Phenytoin sodium Phenobarbitone	200 130	25	14.5	44	33	104	-	360	1.6
13	40 M	Phenytoin sodium Phenobarbitone	300 150	6	16.0	49	33	92	-	490	2.0
14*	35 F	Phenytoin sodium‡ Phenobarbitone	200 200	5	13.8	41	34	93	-	330	1.9
15	44 M	Phenytoin sodium Phenobarbitone	300 150	15	15.7	47	33	95	-	360	1.3
Patients with Symptoms											
16§	30 F	Phenytoin sodium Phenobarbitone	300 150	20	12.9	37	35	97	+	360	1.1
17	56 M	Phenytoin sodium‡ Phenobarbitone	200 200	1	13.8	41	34	94	+	160	1.6
18	37 M	Phenobarbitone†	60	10	16.3	48	34	90	-	420	2.0
19	42 M	Phenytoin sodium Phenobarbitone	600 300	10	15.7	48	33	98	-	440	2.1
20	20 M	Phenytoin sodium‡	300	10	14.4	45	32	90	-	280	1.3
21	54 F	Primidone Phenobarbitone	150 90	8	14.0	42	33	91	-	290	1.9

* Post-traumatic epilepsy. † Also on Trinuride. ‡ Also on primidone. § Very poor diet.

and Mollin, 1957). Hawkins and Meynell (1958) did not find significantly low levels of serum vitamin B₁₂ in their patients. Lees (1961) noted that some patients developed low serum vitamin-B₁₂ levels, and he showed that the absorption of radioactive vitamin B₁₂ improved during treatment with folic acid. The finding of a significant lowering of the level of vitamin B₁₂ in our patients would confirm the suggestion of Lees that a functional change occurs in the gut wall, for in our cases there was no indication of a dietary deficiency in vitamin B₁₂. It is possible either that anticonvulsants affect the absorption of vitamin B₁₂ *per se* or that a deficiency of folic acid impairs vitamin-B₁₂ absorption.

Summary

Haematological studies on a randomly selected group of epileptic patients on anticonvulsants have demonstrated an incidence of macrocytosis in 11% and of low serum folic-acid levels in 37%. Compared with a group of normal control subjects these patients had significantly low levels of serum folic acid and some reduction in serum vitamin B₁₂. This study confirms that the primary cause of these phenomena is the anticonvulsant therapy, but comparison with previous surveys and with a group of less well nourished subjects suggests that

an adequate diet may protect against the occurrence of macrocytosis.

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Amoebiasis—a Diagnostic Problem in Great Britain

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Most clinicians working in a large hospital in this country are unlikely to see more than one or two cases of amoebiasis a year. Between 1938 and 1964 63 patients were given a course of treatment for amoebiasis at the Radcliffe Infirmary, Oxford. In only 19 of these was the diagnosis established by the demonstration of *Entamoeba histolytica* in the stools or in biopsy specimens, but in the remainder a therapeutic trial of amoebicidal drugs was thought to be justified on clinical grounds.

In some patients amoebiasis presents little difficulty in diagnosis, as there is a history of bloody diarrhoea contracted abroad. In others the diagnosis may be missed, either because it is not considered or because the clinical features are unusual.

The present report deals with two cases of amoebic colitis and six cases of hepatic or pleuropulmonary amoebiasis seen here in recent years, all of which were a problem in diagnosis. Two were fatal, and in four others surgical procedures were carried out before the diagnosis was made.

Case 1

A man aged 34 was first seen at the ulcerative colitis clinic in November 1960 with a six-months history of diarrhoea with blood. At sigmoidoscopy the mucosa was hyperaemic and granular and a barium-enema examination was compatible with a diagnosis of ulcerative colitis. He was treated with a nightly rectal infusion of hydrocortisone hemisuccinate 100 mg., and within two weeks symptoms had regressed. In November 1962 there was a recurrence of symptoms with similar appearances at sigmoidoscopy, and a further course of local corticosteroids was given—again with improvement.

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In September 1964 he was admitted to hospital as an emergency because of massive rectal haemorrhage and fainting after two weeks of diarrhoea. Sigmoidoscopy revealed mild but definite inflammation of the rectal mucosa, and treatment with systemic and local corticosteroids was begun. Barium-enema report (Dr. K. Lumsden): "There may be some inflammatory changes in the distal colon. . . . There is a constant filling defect in the caecum which appears to be due to an intraluminal mass of 'polypoid type'." A diagnosis of ulcerative colitis with a carcinoma of the caecum was made and a right hemicolectomy performed. There were several ulcers in the caecum (one of which perforated at the operation) and a mass of granulation tissue; microscopic examination showed deep ulceration with numerous *E. histolytica* in the lamina propria. A course of emetine hydrochloride by intramuscular injection was started on the sixth post-operative day and the corticosteroids were reduced, being finally stopped one week later. When questioned the patient said that he had served with the armed Forces in Burma from 1946 to 1948 but had not had an attack of dysentery at the time.

Sigmoidoscopy three weeks after starting emetine was normal, but three months later, when he was having some looseness of the stools, the mucosa was fragile; stool examination and rectal biopsy were negative for amoebae. In the nine months that have since elapsed he has had no further bowel symptoms.

Case 2

A man aged 56 was admitted to hospital as a surgical emergency in February 1964 with a history of abdominal pain for four weeks and watery diarrhoea without blood for five days. He had lost weight and was known to have had rheumatoid arthritis for 10 years, treated with phenylbutazone. Apart from a visit to Calais in 1946 he had not been abroad. There was generalized guarding of the abdomen with rebound tenderness, most marked in the left iliac fossa. At emergency laparotomy free fluid was found in the