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Reversible absorptive defects in anticonvulsant megaloblastic anaemia

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SYNOPSIS Two cases of anticonvulsant megaloblastic anaemia are described, showing features of unusual interest. Though both cases were apparently deficient in folic acid, the Figlu tests were negative. One patient had an extremely low serum B_{12} concentration apparently associated with defective B_{12} absorption due to deficiency of intrinsic factor, and both showed impaired intestinal absorption of D-xylose. There was, however, no evidence of permanent gastro-intestinal dysfunction, and the absorptive defects disappeared completely after treatment with folic acid.

Possible reasons for the findings are discussed. It is suggested that absorptive defects produced by the drugs may play some part in initiating anticonvulsant megaloblastic anaemia, and that once deficiencies of haemopoietic factors are established, a vicious circle may be set up owing to the effects of these deficiencies on the gastro-intestinal tract.

Since Mannheimer, Pakesch, Reimer, and Vetter (1952) and Badenoch (1954) first suggested a causal relationship, megaloblastic anaemia has become a recognized though infrequent complication of anticonvulsant therapy. The precise mechanism of production of the anaemia remains uncertain. Girdwood and Lenman (1956) suggested that anticonvulsants might act by competitive inhibition of some enzyme system involving folic acid. Positive evidence for this hypothesis is lacking, though it seems that all cases respond to folic acid. The addition of anticonvulsant drugs to microbiological systems has produced no evidence of interference with folic acid metabolism (Christenson, Ultmann, and Roseman, 1957; Klipstein, 1964). Though Klipstein holds that the megaloblastic anaemia is ultimately due to folic acid deficiency, Chanarin and Mollin and their colleagues (Chanarin, Elmes, and Mollin, 1958; Chanarin, Mollin, and Anderson, 1958; Chanarin, Laidlaw, Loughridge, and Mollin, 1960) found evidence of folic acid deficiency in some cases but not in others, and suggested that 'ineffective folic acid metabolism induced by the anticonvulsants will, if continued long enough, result in folic acid depletion of the tissues'. In the few cases in which serum folate concentrations have been measured, they have been low (Klipstein, 1964). It has been suggested that poor diet may be a contributory factor in some cases of megaloblastic anaemia associated with anticonvulsants, and that the anaemia may be precipitated by ascorbic acid deficiency (Newman and Sumner, 1957; Gydell, 1957; Kidd and Mollin, 1957; Flexner and Hartmann, 1960). Intestinal function has not been extensively investigated in the condition. Folic acid absorption tests are usually normal (Chanarin *et al.*, 1958; Hawkins and Meynell, 1958; Klipstein, 1964). Lees (1961) suggested that anticonvulsant drugs might produce a functional change in the gut wall sufficient to produce B_{12} deficiency in some patients, and reported improvement in B_{12} absorption after treatment with folic acid. Jejunal biopsies were normal in three cases (Penny, 1962; Druskin, Wallen, and Bonagura, 1962; Horsfield and Chalmers, 1963). In reports of over 80 cases of anticonvulsant megaloblastic anaemia, the xylose absorption test is mentioned only twice. Penny (1962) found a normal result in his case, while Flexner and Hartmann (1960) found a subnormal xylose excretion but regarded it as of no significance as the serum concentration was just within normal limits.

We report here two cases of anticonvulsant megaloblastic anaemia showing temporary absorptive defects. In one there was impaired B_{12} absorption with a very low serum B_{12} concentration, and in

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both there was marked impairment of xylose absorption. The absorptive defects disappeared after treatment.

METHODS

Serum B_{12} was estimated with *Euglena gracilis*, z strain, by the method of Hutner, Bach, and Ross (1956). Our normal range by this method is 150 to 960 $\mu\mu$g. per ml. Serum folate was estimated with *Lactobacillus casei* (Waters and Mollin, 1961); the normal range is 5·9-21 mμg. per ml. though values of down to 4 mμg. per ml. occur in a variety of pathological conditions not associated with overt deficiency of folic acid. Schilling tests were carried out by giving 0·3 μC^{58} Co B_{12} in 0·5 μg. orally, followed by a flushing dose of 1,000 μg. When necessary, they were repeated after three days with 50 mg. intrinsic factor. With this technique, a 24-hr. urinary excretion of 15% or over is normal; in pernicious anaemia the excretion is less than 7·5% (*e.g.*, Gräsbeck, 1962). The urinary excretion of Figlu was estimated after an oral dose of 15 g. L-histidine. These estimations were kindly performed by Dr. Chanarin by the enzymatic assay; the normal range is 1-17 mg. in 24 hours. Xylose tests were carried out by a standard technique (Benson, Culver, Ragland, Jones, Drummey, and Bougas, 1957); 25 g. of D-xylose was given orally and the urinary excretion measured over the next five hours; the serum concentration was estimated at 90 minutes. Normal urinary excretion is not less than 4 to 4·5 g., and the 90-min. serum concentration is normally more than 30 mg. per 100 ml.

CASE HISTORIES

CASE 1 A 23-year-old man (case no. 93876) was admitted to the National Hospital under the care of Dr. Michael Kremer on 19 November 1963 with symptoms of anaemia. He had suffered from idiopathic epilepsy from the age of 16 onwards. This was always difficult to control and required unusually large doses of anticonvulsant drugs. In 1958, two years after starting treatment with phenobarbitone 130 mg. and phenytoin 200 mg. daily, he was admitted to another hospital with a severe macrocytic anaemia (Hb 6·8 per 100 ml.), a megaloblastic bone marrow, and a serum B_{12} level of 20 $\mu\mu$g./ml. Free acid was present in the stomach. The anticonvulsants were withdrawn, and a rapid haematological response to B_{12} therapy was obtained. Folic acid was not used at this time and B_{12} therapy was stopped after a few months. A year after treatment with phenobarbitone had been recommenced he again had a megaloblastic anaemia with a haemoglobin of 8·0 g. per 100 ml., which responded to B_{12} and folic acid while phenobarbitone was continued. From then until his admission to the National Hospital two years later he had been taking 325 mg. phenobarbitone daily without folic acid or other haematinics. His usual diet contained normal amounts of meat and a good deal of fish and chips. He disliked potatoes unless fried, and disliked fresh vegetables and fruit.

When seen on admission he was pale and had recently lost a stone in weight. The tongue was smooth but not sore and there had been no disturbance of bowel habits. The tip of the spleen was palpable. Results of the relevant investigations were as follows: Hb 7·0 g. per 100 ml.; R.B.C. 1·9 million per c.mm., P.C.V. 20%, M.C.H.C. 35%, M.C.V. 105 cμ; W.B.C. 3,600 per c.mm. (polymorphs 48%, lymphocytes 46%, monocytes 3%, eosinophils 3%); platelets 123,000 per c.mm. The red cells were macrocytic with marked anisocytosis and poikilocytosis. The polymorphs showed hypersegmentation. The bone marrow was active and hypercellular with gross megaloblastic changes and many giant metamyelocytes. A test meal with 0·5 mg. histamine showed no free acid. A random sample of serum iron was 205 μg. per 100 ml. The serum folate level was 4·0 mμg. per ml. No Figlu was detected in the urine. The serum B_{12} level was 10 $\mu\mu$g. per ml. A Schilling test showed excretion of 7% of the dose; after repetition with intrinsic factor, the excretion rose to 14%. An ascorbic acid saturation test (Varley, 1962) showed saturation on the fifth day. The mean faecal fat excretion on a normal ward diet was 1·6 g. per day and 1·2 g. per day in two three-day collections. The five-hour xylose excretion was 3·5 g. and the 90-min. serum level 26 mg. per 100 ml. A glucose tolerance curve after 50 g. orally gave 91 (fasting), 105, 122, 118, and 114 mg. per 100 ml. (30-min. intervals after dose). The results of a barium meal and follow-through were normal. Liver function tests and plasma proteins were normal. Blood urea was 30 mg. per 100 ml.

Treatment was started with vitamin B_{12} 1,000 μg. intramuscularly daily, on 1 December. The course of events is shown in Figure 1. There was a reticulocyte response reaching 9% on the seventh day but there was no significant alteration in haemoglobin or red cell count. Treatment with folic acid (30 mg. intramuscularly t.d.s. for two days, followed by 10 m.g. b.d. orally), was started on 10 December and produced a reticulocyte response reaching 20% on the fourth day, followed by a rise in Hb; oral iron (ferrous gluconate 325 mg. t.d.s.) was added on 23 December. Phenobarbitone was continued throughout. Three weeks after starting treatment with folic acid, the Hb had risen to 11·6 g. per 100 ml. (R.B.C. 3·96 million per c.mm., P.C.V. 40%, M.C.H.C. 30%, M.C.V. 10 cμ.; W.B.C. 4,900, with polymorphs 51%, lymphocytes 48%, eosinophils 1%). The bone marrow was normoblastic. At this stage the Schilling test was repeated, and 22% of the dose was excreted without intrinsic factor, a result well within the normal range. A test meal showed free acid in the fasting juice without histamine stimulation. The urinary excretion of D-xylose was now 5·1 g. in five hours and the 90-min. serum concentration 42 mg. per 100 ml. Successive E.E.G.s throughout his stay in hospital showed a diminution in epileptic activity as the anaemia was corrected. Treatment with iron, B_{12}, and folic acid were continued, and on 16 March 1964 the Hb was 15·4 g. per 100 ml. and the red and white cells normal.

CASE 2 A 25-year-old woman (case no. A/14108) was admitted to the National Hospital on 10 October 1963 under Professor R. W. Gilliatt. She had had attacks of temporal lobe epilepsy since the age of 10, for which she had been on continuous anticonvulsant therapy with a

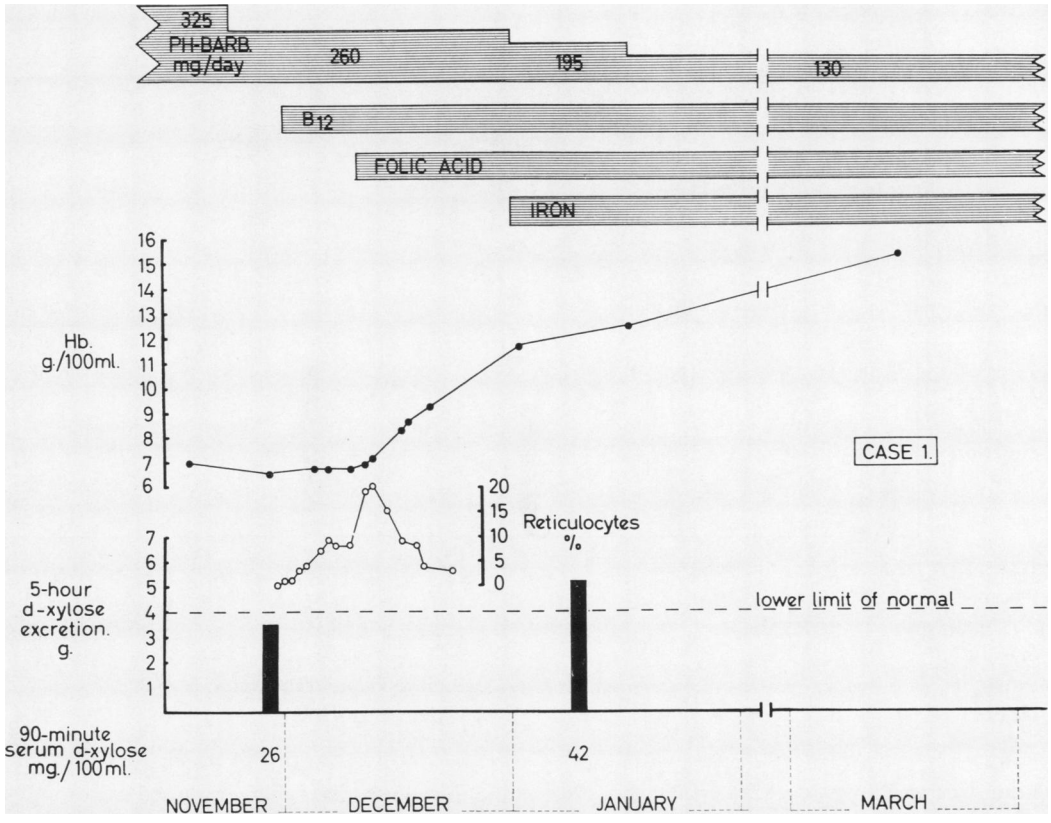

FIG. 1. *Response to treatment and improvement in xylose tests in case 1.*

combination of phenobarbitone and epanutin (phenytoin sodium) in varying doses. For a year before admission she had also had trinuride (phenylethylacetylurea 200 mg., phenytoin sodium 40 mg., phenobarbitone 15 mg.) t.d.s. Two years earlier she had had a psychotic illness from which she had recovered, but had subsequently been taking stelazine (trifluoperazine) 10 mg. t.d.s. Her usual diet contained reasonable quantities of meat, fish, eggs, and milk, but like the first patient she disliked green vegetables, salads, and fresh fruit, and ate very little of these.

On investigation she was found to have pathological calcification in the right temporal lobe, associated with a spike focus in the same region on her E.E.G. General examination was normal, though she was rather pale and was found to be mildly anaemic. Results of haematological and other relevant investigations were as follows: Hb 10·8 g. 100 ml., R.B.C. 3·73 million per c.mm., P.C.V. 39%, M.C.H.C. 29%, M.C.V. 105 cμ.; W.B.C. 6,900 per c.mm. (polymorphs 70%, lymphocytes 26%, monocytes 3% eosinophils 1%); platelets 210,000 per c.mm. The red cells showed anisocytosis and many were macrocytic. The bone marrow showed early megaloblastic changes. An augmented histamine test meal showed free acid. The direct Coombs test was negative. A random sample showed that the serum iron level was 150 μg. per 100 mg. The serum B_{12} was 130 $\mu\mu$g. per ml. This estimation was repeated on two subsequent occasions before treatment with results of 210 and 220 $\mu\mu$g. per ml. A Schilling test was not done until after treatment. The serum folate was estimated twice before treatment with results of 6·0 and 4·5 mμg. per ml. The Figlu excretion was 11 mg. (normal). The urinary xylose was 2·7 g. in five hours, and the 90-min. serum level 25 mg. per 100 ml. This low result was confirmed on two other occasions before treatment. Mean faecal fat excretion on a normal ward diet was 3 g. per day (three-day collection). The glucose tolerance test after 50 g. orally showed 61 (fasting), 114, 134, 134, and 106 mg. per 100 ml. (30-min. intervals after dose). A barium meal and follow-through were normal. Serum proteins and liver function tests were normal. The blood urea was 30 mg. per 100 ml. From 1 October 1963 to 1 January 1964, while she was undergoing neurological and haematological investigations, she remained on a normal ward diet. Her anticonvulsant medication was continued in the form of phenytoin sodium 100 mg. t.d.s.

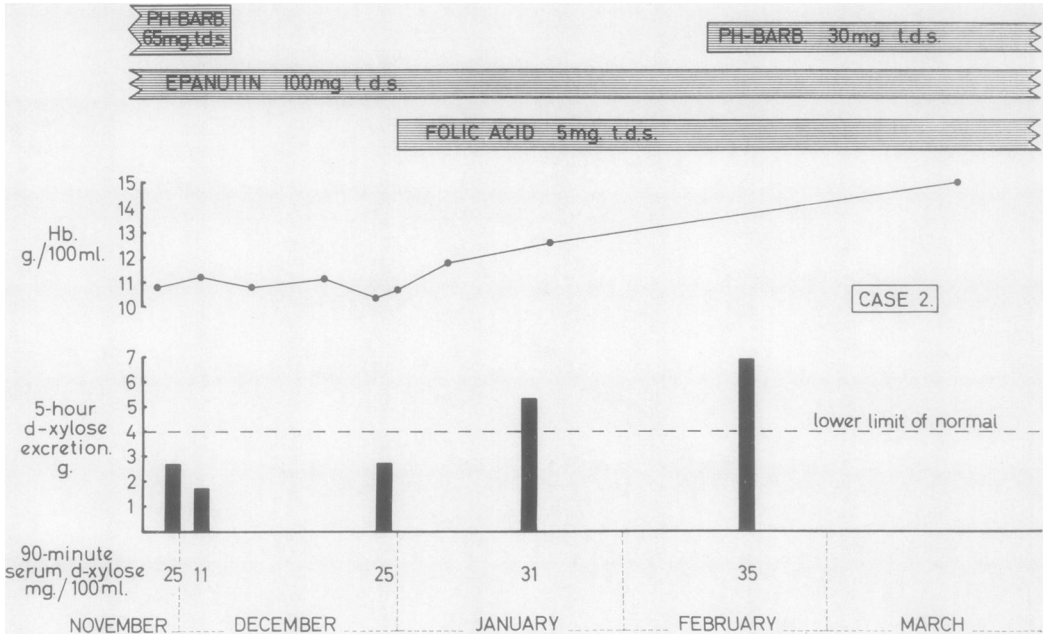

FIG. 2. *Response to treatment and improvement in xylose tests in case 2.*

and phenobarbitone 65 mg. t.d.s. until 6 December 1965, when the phenobarbitone was discontinued. There was no change in reticulocytes or haemoglobin throughout this period. Treatment with folic acid 5 mg. t.d.s. was started on 1 January 1964. Following this there was a steady rise in Hb and an improvement in the results of the results of the xylose tests (Fig. 2). On 12 December 1964, phenobarbitone 30 mg. t.d.s. was re-introduced as she continued to have occasional fits while on epanutin alone. A Schilling test was done on 25 February 1964 about eight weeks after treatment had begun; 17% of the dose was excreted in 24 hours without intrinsic factor. On 18 March, the Hb was 15 g. per 100 ml. and the red and white cells appeared normal.

DISCUSSION

The diagnosis of megaloblastic anaemia due to anticonvulsants rests partly on the exclusion of the other causes of megaloblastic anaemia. Some features of case 1, the very low serum B_{12}, the absence of free gastric acid, and the subnormal Schilling test with some response to intrinsic factor, at first suggested pernicious anaemia, but this was excluded by the presence of free gastric acid and a normal Schilling test after treatment. In both cases the results of the initial xylose test suggested the possibility of some malabsorptive condition, but the faecal fat was well within normal limits, barium meals were normal, and the xylose tests rapidly returned to normal after treatment, so that an underlying intestinal defect such as idiopathic steatorrhoea seems unlikely. Both patients had been on diets that were rather poor in some respects, but not so inadequate that dietary deficiencies alone would be expected to produce megaloblastic anaemia. Consequently in both cases the magaloblastic anaemia was considered to be due to the anticonvulsant therapy. As examples of this condition, the cases present interesting features.

Though the serum B_{12} concentration is occasionally subnormal in anticonvulsant megaloblastic anaemia (Kidd and Mollin, 1957; Flexner and Hartmann, 1960; Klipstein, 1964), we believe that the concentration of 10 $\mu\mu$g. per ml. in case 1 is the lowest ever recorded in the condition. It may have been the result of impaired absorption, as suggested by the Schilling test. Primary folate deficiency can cause a decline in serum B_{12} (Narayanan, Shenoy, and Ramasarma, 1956; 1957; Mollin and Ross, 1957; Chanarin and Bennett, 1962; Chanarin, 1964) but such low levels have not been reported in association with this. In case 2, the serum B_{12} was slightly subnormal on one occasion only.

Both cases had low, though not grossly subnormal, serum folate concentrations, both responded to folic acid, and case 1 responded only very poorly to

B_{12}. These features suggest deficiency of folic acid, yet both cases gave a negative Figlu test, and in case 1 Figlu excretion was undetectable. Failure to excrete excess Figlu with a histidine load is very unusual in definite megaloblastosis due to folate deficiency alone, though it does occur in some cases of megaloblastic anaemia of pregnancy, and has been noted even when the anaemia is severe. In this condition, the phenomenon is partly due to slow absorption of histidine (Chanarin, Rothman, and Watson-Williams, 1963), and possibly this contributed to the results in our cases. Negative Figlu tests have been found in two other cases of anticonvulsant megaloblastic anaemia (Chanarin, 1964), but in both of these, the anaemia was very slight.

The results of the xylose tests, and of the Schilling test in case 1, are particularly interesting. The main causes of subnormal excretion of D-xylose after oral administration are reduced intestinal absorption and reduced renal excretion. In both our cases, the results indicated impaired absorption, as the serum concentrations were also subnormal. Had renal impairment been responsible, the serum levels should have been high. Within a few weeks of starting treatment, and before the haemoglobin had reached its maximum level, the absorptive defect for xylose disappeared. In case 1, the glucose tolerance test carried out before treatment was also compatible with malabsorption of monosaccharides. The urinary excretion of radioactivity in the pre-treatment Schilling test in case 1 indicated that B_{12} absorption was also reduced, apparently owing to deficiency of intrinsic factor. After treatment the result was completely normal without giving intrinsic factor, and free gastric acid was found without histamine stimulation.

The existence of reversible disorders of gastrointestinal function in these cases of anticonvulsant megaloblastic anaemia raises the question of their relationship to the condition. One possibility is that they were secondary to disturbed metabolism of folic acid induced by the drugs. It is unlikely that they were the results of anaemia *per se* (in case 2 the anaemia was very mild) but it would not be surprising if multiple defects of gastrointestinal function were to result from deficiency or disordered metabolism of folic acid or vitamin B_{12}, as both these factors are necessary for nucleoprotein synthesis and normal cell maturation, and the rate of renewal of the mucosal cells of the alimentary tract is very high. Animal experiments have shown that folic acid antagonists cause degeneration of the intestinal mucosal cells (Jacobson, 1954) and malabsorption of xylose (Butterworth, Perez-Santiago, Martinez de Jesus, and Santini, 1959; Zamcheck, 1960). The literature contains many scattered reports suggesting that in megaloblastic anaemias there may be absorptive defects involving galactose (Singer and Wechsler, 1934), D-xylose (Helmer and Fouts, 1937; Butterworth *et al.*, 1959; Bezman, Kinnier, and Zamcheck, 1959; Wormsley, 1963), iodide (Heath and Fullerton, 1935; Bezman *et al.*, 1959), amino-acids (Erf and Rhoads, 1940), and fat (Mollin, Booth, and Baker, 1957; Lambert, Prankerd, and Smellie, 1961), while Schloesser and Schilling (1963) have reported a case of megaloblastic anaemia due to dietary deficiencies in which there was a reversible absorptive defect for vitamin B_{12}. Consequently it is possible that in both our cases malabsorption of xylose was the result of interference with folic acid metabolism by the anticonvulsants, and this may also have accounted for the impaired B_{12} absorption in case 1, probably mainly by reducing secretion of intrinsic factor. The development of B_{12} deficiency may have aggravated the absorptive defects.

A second possibility is that the anticonvulsants were directly responsible for the disturbances of gastrointestinal function, and that this effect was overcome by treatment. An inhibitory effect of anticonvulsants on the absorptive mechanisms for monosaccharides is suggested by the observation that phenobarbitone reduces the absorption of glucose *in vitro* (Riklis and Quastel, 1958). Our own preliminary experiments have confirmed this, and show that xylose absorption is also inhibited. Whether anticonvulsant drugs have any effect on the absorption of folic acid or vitamin B_{12} is unknown. Though the results of folic acid absorption tests using free pteroylglutamic acid are usually normal in megaloblastic anaemia due to anticonvulsants, there is evidence suggesting that these tests may not be a reliable guide to the absorption of dietary folic acid, which occurs largely in bound forms (Girdwood, 1960; 1964; Druskin *et al.*, 1962; Cooke, Fone, Cox, Meynell, and Gaddie, 1963), and we feel that the possibility that drug-induced absorptive defects may play some part in initiating this anaemia has not yet been satisfactorily excluded.

In conclusion, we should like to draw special attention to the following points: (1) that the Figlu test may be negative in anticonvulsant megaloblastic anaemia; (2) that in one of our cases the initial laboratory findings closely resembled those in pernicious anaemia; (3) that in any megaloblastic anaemia, absorptive defects are likely to occur, and subnormal xylose absorption is not a reliable indication of permanently impaired intestinal function; and (4) that whenever deficiences of folic acid or vitamin B_{12} have arisen in some way, it is possible that a vicious circle may be set up, owing to the effects of deficiencies of these factors on intestinal absorption.

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