

Changes in serum vitamin B₁₂ levels in patients with megaloblastic anaemia treated with folic acid

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SYNOPSIS In 21 patients with megaloblastic anaemia associated with the tropical malabsorption syndrome serum vitamin B₁₂ levels have been measured serially before and during parenteral folic acid therapy.

In 11 patients there was a significant rise in serum B₁₂ levels, reaching a peak three to 17 days after starting the folic acid. In three of these cases, absorption of labelled B₁₂ was the same before and after the administration of folic acid. In two cases there was a fall in serum B₁₂ levels and in seven there was no change. In one case small amounts of folic acid produced no change in serum B₁₂ levels, whereas larger doses produced a prompt but transient rise.

A rise in the serum vitamin B₁₂ levels in patients treated with folic acid has been noticed by several workers (Narayanan, Shenoy, and Ramasarma, 1956, 1957; Narayanan, 1961; Mollin and Ross, 1957). It is the purpose of this communication to report further observations on the effect of folic acid therapy on the serum vitamin B₁₂ levels of patients with megaloblastic anaemia associated with the tropical malabsorption syndrome.

MATERIALS AND METHODS

Serum vitamin B₁₂ estimations were performed by the method of Ross, Hutner, and Bach (1957) using *Euglena gracilis* Z strain. Radioactive vitamin B₁₂ absorption tests were carried out by the faecal excretion method of Heinle, Welch, Scharf, Meacham, and Prusoff (1952) using 1 µg. vitamin B₁₂ labelled with Co⁵⁸ (obtained from the Radiochemical Centre, Amersham, England) and carbamylcholine chloride as a stimulant to intrinsic factor secretion (Baker and Mollin, 1955).

Twenty-one patients suffering from the malabsorption syndrome or 'idiopathic tropical steatorrhoea' and associated megaloblastic anaemia (Baker 1957, 1958) were studied as hospital in-patients. In each case patients were kept on the same diet as they had been having before admission, the patients' relatives supplying their own food from home. In every case there was a control period of at least 10 days in hospital before any therapy was begun, and subsequently daily intramuscular injections of folic acid were given. In Cases 1 to 20, 15 mg. a day was given for 20 to 30 days. In Case 21, 0.2 mg. was given daily for 22 days and then the dose was increased to 15 mg. a day for 28 days. No other specific therapy was given

during the course of the study. The serum vitamin B₁₂ levels in the plasma were estimated at regular intervals.

RESULTS

SERUM B₁₂ LEVELS The changes in the serum B₁₂ levels are shown in Fig. 1. In 11 patients (Fig. 1, Cases 1 to 11) there was a significant rise in serum B₁₂ levels reaching a maximum in three to 17 days. In Case 1 this rise in serum levels was sustained throughout the period of observation. Case 7 left hospital on the fourth day of folic acid therapy. In all the other cases the rise in serum levels was subsequently followed by a fall with a tendency to return to pre-treatment levels.

In two patients there was a slight fall (Fig. 1, Cases 12 and 13), and in seven patients there was no significant change in serum vitamin B₁₂ levels (Fig. 1, Cases 14 to 20).

In Case 21 there was no change in serum B₁₂ level when a dose of 0.2 mg. of folic acid was given daily, but a transient rise was observed when the dose was increased to 15 mg. a day (Fig. 2).

VITAMIN B₁₂ ABSORPTION In three patients (Cases 1, 6, and 8) in whom folic acid produced a rise in serum B₁₂ levels, B₁₂ absorption before and after folic acid was measured. In each case B₁₂ absorption was defective, and there was no significant improvement following folic acid administration (Fig. 3).

HAEMATOLOGICAL RESPONSE In Cases 1 to 20 after the administration of folic acid the marrow reverted

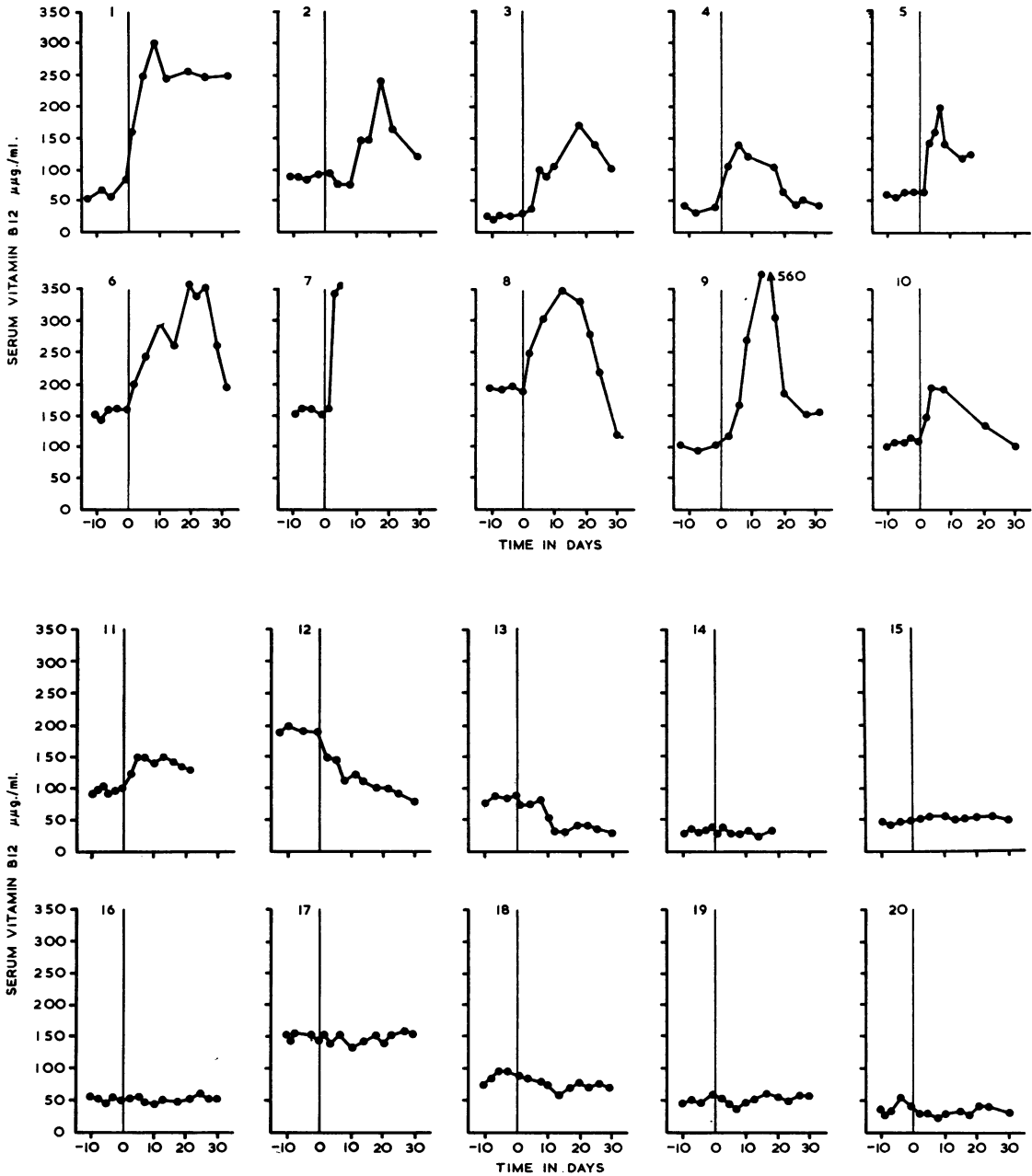


FIG. 1. Serum B₁₂ levels in µg./ml. in Cases 1 to 20 given daily injections of 15 mg. of folic acid from day 0 onwards.

to a normoblastic pattern and there was a reticulocytosis, but in many this was suboptimal, and in these cases the subsequent rise in red cells and haemoglobin was also suboptimal. There was no correlation between the haematological response

and the degree of change in serum B₁₂ levels. In Case 21 there was no haematological response to 0.2 mg. of folic acid but on increasing the dose to 15 mg. there was a bone marrow response followed by a suboptimal rise in red cells and haemoglobin.

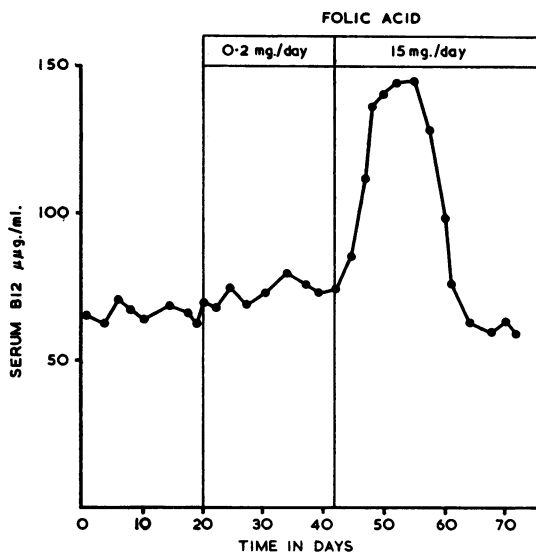


FIG. 2. Serum B_{12} levels in $\mu\text{g./ml.}$ in Case 21 given daily injections of folic acid; on days 20 to 42 in a dose of 0.2 mg.; on days 43 to 70 in a dose of 15 mg.

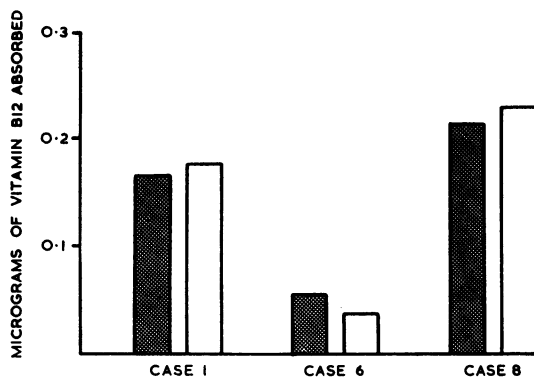


FIG. 3. The amount of vitamin B_{12} absorbed from a test dose of 1 $\mu\text{g.}$ of B_{12} in Cases 1, 6, and 8. The shaded column represents the amount absorbed when the test was carried out in the control period, and the open column when carried out after one week of folic acid therapy.

DISCUSSION

It is interesting that 11 of the first 20 patients showed a significant rise in serum B_{12} levels, while the other nine showed no change or a slight fall. The reason for this difference in response to folic acid administration is not clear. There was no detectable clinical

or haematological difference between the patients in whom there was a rise in B_{12} levels and ones in which there was no rise, except that the mean pretreatment serum B_{12} level was higher in the former group (98.5 $\mu\text{g./ml.}$) than in the latter (77.3 $\mu\text{g./ml.}$). However, Case 3, in which there was a rise in B_{12} levels, had pre-treatment serum levels of 20 to 25 $\mu\text{g./ml.}$, a level as low as any in the group which did not show a rise. Narayanan *et al.* (1957) have suggested that those with greater body stores are those more likely to show a rise in serum levels. It may well be that pre-treatment serum levels do not always accurately indicate the level of body stores, but at least one patient (Case 1) was known to have had a B_{12} absorptive defect for five years and his body stores must therefore have been low; nevertheless he showed a prompt rise in serum levels after the administration of folic acid.

The maximum rise in serum vitamin B_{12} levels observed ranged from 100 to 460 $\mu\text{g./ml.}$ If we assume that the total plasma volume is 3 litres this means a total increase in the plasma B_{12} of about 1.5 $\mu\text{g.}$ This could come about either by an improved absorption of B_{12} from the gastrointestinal tract or by an internal shift of vitamin B_{12} from the tissue stores to the plasma.

The fact that in three cases the absorption of radioactive vitamin B_{12} before and after the administration of folic acid was unchanged suggests that improved absorption is not the explanation for the rise in serum levels.

The initial rise in serum B_{12} levels could be explained on the basis of mobilization of B_{12} from 'labile' body stores, presumably in the liver, and the subsequent fall in serum levels occurring when these labile stores were exhausted. The patient described by Mollin and Ross (1957) did not show a subsequent fall in B_{12} levels during the period of study. It may be that this patient's body stores were high. Such an explanation, however, does not seem very likely in our Case 1, who had a long-standing absorptive defect.

Even in B_{12} -depleted individuals the amount of B_{12} needed to raise serum levels will only be a small fraction of the total amount remaining in the body. One would therefore not be likely to be able to detect this change in body stores by currently available methods.

It is interesting that in Case 21 small doses of folic acid failed to cause any increase in serum B_{12} levels, whereas larger doses caused a prompt though transient rise. This suggests that there may be a critical amount of folic acid needed to produce this effect.

Wilson and Pitney (1955) found that in monkeys fed on a folic acid-deficient diet the serum B_{12} levels

fell, and in two out of three such animals given folic acid there was a rise in serum B₁₂ levels followed by a fall. They suggested, without any direct evidence, that the folic acid deficiency depressed B₁₂ absorption which was improved on giving folic acid.

Cox, Meynell, Gaddie, and Cooke (1959), in 13 patients with iron-deficiency anaemia and low serum B₁₂ levels, found a rise in serum B₁₂ following iron administration. The peak rise, however, in their cases was much later than in our patients given folic acid, and the mechanism involved may be quite different.

The changes in serum B₁₂ levels after folic acid administration underline the difficulties in diagnosis which may occur when folic acid is given to patients before adequate investigations have been undertaken. The use of folic acid may convert a low serum B₁₂ level, even in a patient with depleted B₁₂ stores, into a normal or near normal serum level, thus masking the diagnosis. Further, random serum B₁₂ estimations may be misleading in assessing the B₁₂

status of an individual, unless the folic acid status of the patient is also known.

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