Vitamin-B_{12} Status of Patients on Long-term Metformin Therapy

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Summary

Vitamin-B_{12} malabsorption has been found in 21 (30%) of 71 diabetic patients taking long-term metformin therapy in addition to dietary management. The patients with evidence of B_{12} malabsorption had significantly lower haemoglobin levels (and significantly higher serum folic acid levels) than those with normal B_{12} absorption. Steatorrhoea was found in only one patient. Stopping metformin therapy resulted in reversion of B_{12} absorption to normal in most patients examined. Four patients with B_{12} malabsorption were found to have pathologically low serum B_{12} levels. The causes and implications of these findings are discussed and it is concluded that all patients on long-term metformin therapy should have annual serum B_{12} estimations.

Introduction

The biguanides have been used in the treatment of diabetes mellitus since 1957, when they were first reported to have an effective hypoglycaemic action in man (Ungar *et al.*, 1957). Loss of weight has been noted to be one of the effects of this therapy, but the cause is disputed (*Journal of the American Medical Association*, 1970), though intestinal malabsorption is certainly an important factor (Czyzyk *et al.*, 1968; Stowers and Bewsher, 1969). Berchtold *et al.* (1969) reported malabsorption of vitamin B_{12} in patients who had been treated with metformin for two to three months, but their results were not confirmed when other biguanides were used (Willms and Creutzfeldt, 1970).

This paper reports a study designed to investigate B_{12} absorption and serum B_{12} status in patients on long-term metformin therapy.

Patients and Methods

All diabetic patients treated with metformin for more than two years who were reviewed at the diabetes clinic of the Royal Victoria Hospital during a three-month period (1970-1) were investigated. B_{12} absorption in these patients was compared with a group of 19 diabetic patients who had been treated with chlorpropamide for more than four years (Table I).

Standard laboratory methods were used for the determination of haemoglobin, serum iron, serum B_{12} (*Lactobacillus leichmannii*), folic acid, faecal fat, and serum carotene.

B_{12} absorption was measured by a double isotope technique (Bell *et al.*, 1965) which depends on the simultaneous administration of B_{12}, labelled with two isotopes of cobalt (^{58}Co and ^{57}Co), the ^{57}Co being first bound to intrinsic factor of human gastric juice. Patients with pernicious anaemia will preferentially absorb the intrinsic-factor-bound vitamin B_{12}. The intrinsic factor assay was based on the technique of Ardeman and Chanarin (1963). A standard 25-g D-xylose test was used and the results were expressed as a percentage of the dose excreted in five hours. A ^{14}C tripalmitate absorption test was used. This test depends on the excretion of the radioactive carbon in the expired CO_2 after an oral dose of ^{14}C tripalmitate and has been found in this department to be an accurate test of fat absorption (Bhatia *et al.*, 1969).

TABLE I—*Groups of Patients Studied (Mean ± S.E. Mean)*

^{57}Co B_{12} Absorption	Age	Daily Hypoglycaemic Dose	Years on Hypoglycaemic Drug	Weight Change (lb.)
		Metformin Patients		
Normal Patients N = 50 ..	61·7 ± 1·36	1·75 ± 0·1 (g Metformin)	4·8 ± 0·3	−3·5 ± 2·6
Abnormal Patients N = 21 ..	63·3 ± 1·95	1·97 ± 0·15 (g Metformin)	4·6 ± 0·37	−6·53 ± 2·68
Significance (Student's *t* test)	*t* = 0·64 P>0·05	*t* = 1·6 P>0·05	*t* = 0·42 P>0·05	*t* = 0·66 P>0·05
		Chlorpropamide Patients		
N = 19 ..	67·4 ± 1·3	302·6 ± 34·25 (mg chlorpropamide)	7·0 ± 0·8*	+5·85 ± 2·8*

*Significantly different from metformin patients (P<0·05).

Results

Tests of Malabsorption.—Seventy-one patients who had been taking metformin for a mean period of 4·6 years (S.E. of mean 0·35) were examined. Twenty-one (30%) were found to have abnormally low B_{12} absorption (Fig. 1). On the basis of these results the patients were divided into normal and abnormal groups (Table I). Those with normal B_{12} absorption had a mean age of 61·7 years which was not significantly different from that of the B_{12} malabsorption patients (mean age 63·3 years) (P>0·05). The mean daily metformin dose of the patients with B_{12} malabsorption was 1·97 g and of the normal absorption group 1·75 g. This difference was not significant (P>0·05). Though the B_{12} malabsorption group of patients had a mean weight loss from the onset of metformin therapy of 6·53 lb (2·96 kg), which was greater than the mean loss of 3·5 lb (1·59 kg) sustained by the normal B_{12} absorption group, this difference was not significant (P>0·05). The chlorpropamide group had been treated for a mean of seven years, which was a significantly longer period than the metformin-treated patients (P<0·05), and the weight increase in these patients of 5·85 lb (2·65 kg) was significant compared with the weight loss sustained by the patients on metformin (P<0·05). The blood urea was normal in all the patients with B_{12} malabsorption. The results of several blood tests carried out on the patients on long-term metformin therapy are shown in Table II. The mean haemoglobin, serum B_{12}, carotene, and iron levels were all lower in the patients with abnormal B_{12}

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absorption, though only the haemoglobin difference reached significance ($P<0.02$). The serum folic acid was significantly higher in the patients with B_{12} malabsorption ($P<0.05$).

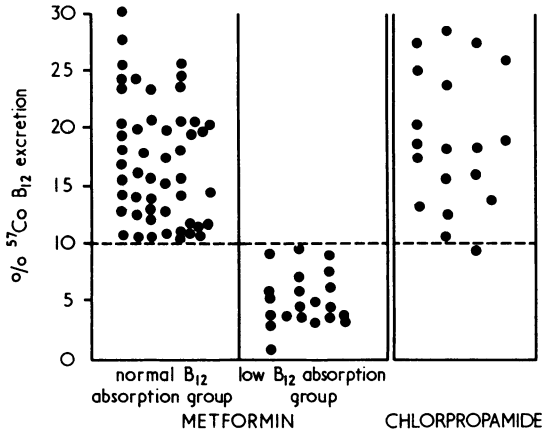

FIG. 1—Results of ^{57}Co B_{12} absorption tests.

TABLE II—*Investigation Results of Patients on Long-term Metformin (Mean ± S.E. of Mean)*

^{57}Co B_{12} Absorption	Hb (g/100 ml)	B_{12} (pg/ml)	Folic Acid (µg/ml)	Carotene (µg/100 ml)	Serum Iron (µg/100 ml)
Normal. N = 50 ..	14·5 ± 0·22	527·1 ± 30·5	7·25 ± 0·5	140·0 ± 11·7	91·1 ± 5·9
Low. N = 21 ..	13·6 ± 1·56	391·2 ± 57·5	9·6 ± 1·6	105·05 ± 14·6	79·5 ± 6·1
Student's *t* test ..	*t* = 2·74 P<0·02	*t* = 1·93 P>0·05	*t* = 2·44 P<0·05	*t* = 1·8 P>0·05	*t* = 1·2 P>0·05

Vitamin-B_{12} Deficiency.—Four patients were found to have evidence of B_{12} deficiency. Three had pathologically low B_{12} levels and the fourth had a low normal level with macrocytosis on the peripheral blood film which disappeared after B_{12} therapy. Details of these four patients are shown in Table III. None of the 50 patients on long-term metformin with normal B_{12} absorption had low serum B_{12} levels. The number of patients with the low B_{12} levels in the B_{12} malabsorption group is more than would be expected by chance ($\chi^2 = 5.58$, $P<0.02$).

Fat Absorption.—Only one patient with B_{12} malabsorption had abnormally high faecal fat excretion (35 g/3 days) and this patient also had a very low ^{14}C tripalmitate absorption test ($13.5 \times 10^{-4}\%$). When re-examined four weeks after cessation of metformin, ^{14}C tripalmitate had become normal ($26 \times 10^{-4}\%$) and faecal fat excretion was 3·9 g in a three-day collection. Only one other patient had a marginally low ^{14}C tripalmitate test.

D-*Xylose.*—Six of the nine patients studied had abnormal D-xylose tests (Fig. 2).

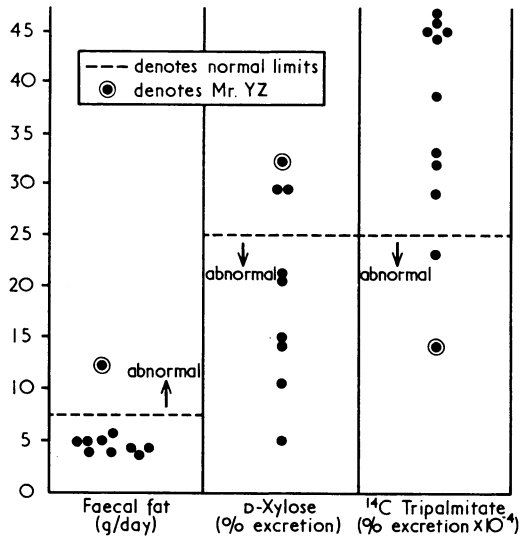

FIG. 2—Results of tests of malabsorption in patients with ^{57}Co B_{12} malabsorption.

Radiological Examination of the Bowel.—Eleven patients were examined and showed no evidence of ileal abnormality.

Intrinsic Factor.—Six patients with B_{12} malabsorption had intrinsic factor measured after a pentagastrin test meal (Table IV). Two were found to have achlorhydria and undetectable intrinsic factor. Intrinsic factor, however, did not improve their B_{12} absorption.

TABLE IV—*Patients on Metformin with Low ^{57}Co B_{12} Absorption*

Case No.	% ^{57}Co B_{12} Excretion (+ I.F.)	Pentagastrin Test Meal (mEq Acid in 1st hour)	Intrinsic Factor (U/ml)
1	10·3	14·3	44·5
2	5·0	Nil	Nil
3	9·1	12·5	46·6
4	7·4	Nil	Nil
5	5·8	26·0	76·0
6	7·2	19·5	44·5

Vitamin-B_{12} Absorption after Antibiotic Therapy.—Five patients with B_{12} malabsorption had a seven-day course of tetracycline but this did not lead to any improvement in the B_{12} absorption on repeat testing (Fig. 3).

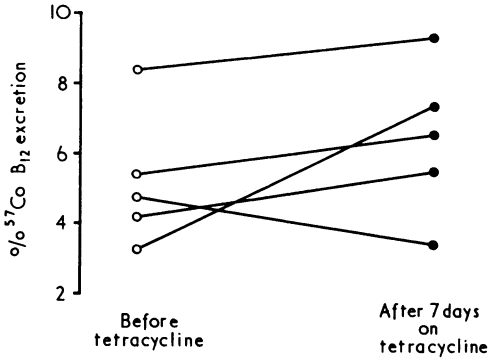

FIG. 3—Change in ^{57}Co B_{12} absorption after tetracycline.

TABLE III—*Details of Patients with Low B_{12} Levels*

Age	% ^{57}Co B_{12} Excretion (+ I.F.)	B_{12} (pg)	Folic Acid (µg)	Hb (g)	Carotene (µg/100 ml)	Iron (µg)	Faecal Fat (g/day)	^{14}C Tripalmitate (% Excretion × 10^{-4})	D-Xylose (% Excretion)	Metformin (g/24 hr)
57	3·5	100	7	12·2	100	70	2·6	46	17	3·0 for 6 years
73	8·6	50	16	14·2	231	110	—	—	21	1·0 for 6 years
65	1·4	125	14	12·3	61	51	<3	32	—	1·5 for 5 years
65	3·3 (14·8)	190	6	15·4*	131	90	11·7 (1·3)	13·5 (25·9)	32	1·5 for 4 years

*Macrocytosis.
Results in parentheses—4 weeks after cessation of metformin.

Cessation of Metformin Therapy.—Seven patients with abnormal B_{12} absorption had their oral hypoglycaemic treatment changed to chlorpropamide. Within 28 days the B_{12} absorption was retested and in all but one it had returned to normal (Fig. 4).

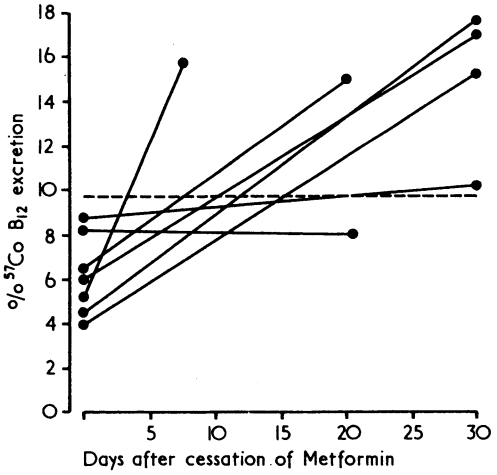

FIG. 4—Change in ^{57}Co B_{12} absorption after substitution of chlorpropamide for metformin in patients with ^{57}Co B_{12} malabsorption.

Discussion

Berchtold *et al.* (1969) found pathologically low levels of B_{12} absorption in patients who had been treated with metformin for up to three months. Willms and Creutzfeldt (1970) found no evidence of B_{12} malabsorption in 15 patients who had been treated with buformin for 27 months and there was no significant change in the absorption of B_{12} when phenformin was substituted over a four-week period. The results presented in this paper show that B_{12} malabsorption is a common finding in patients on long-term metformin therapy and is of clinical importance as 4 of the 21 patients with B_{12} malabsorption had pathologically low B_{12} levels within six years of starting therapy.

The cause of B_{12} malabsorption is not known. It is not due to lack of intrinsic factor, as only two of the six patients examined had achlorhydria and undetectable intrinsic factor (Table IV). Further, the addition of intrinsic factor to the ^{57}Co B_{12} did not return the B_{12} absorption to normal. Achlorhydria and very low intrinsic factor levels (<1% of the normal secretion) have been found with normal B_{12} absorption (Ardeman and Chanarin, 1965), so that the "absent" intrinsic factor found in two of our patients probably reflects the insensitivity of the intrinsic factor assay.

The B_{12} malabsorption cannot be attributed to the small-bowel bacterial colonization found occasionally in diabetics (Goldstein and Wirts, 1970) as the B_{12} absorption of the five patients investigated after tetracycline therapy remained abnormal (Fig. 3). B_{12} is actively absorbed from the distal ileum (Chanarin, 1969) and disease in this area may result in B_{12} malabsorption. However, radiological examination of the ileum was normal in the 11 patients studied.

It remains to be shown whether the malabsorption of B_{12} is due to competitive inhibition by metformin in the distal ileum or whether the enzyme system involved in the active absorption of B_{12} is inactivated by the drug. The fact that almost all the B_{12} absorption tests became normal after stopping metformin for periods of 7 to 28 days suggests that one of these mechanisms may be involved.

The significantly higher folic acid levels found in the patients with B_{12} malabsorption probably reflects lower serum B_{12} levels in this group (Table II). A proportion of patients with pernicious anaemia have been shown to have relatively high serum folate levels (Herbert *et al.*, 1960; Waters and Mollin, 1961).

Fat malabsorption was suggested but not proved by Berchtold *et al.*, (1969) in patients on metformin. Only one of our patients was found to have definite fat malabsorption so that the mechanism of the B_{12} malabsorption does not appear to be related to this finding. This is further supported by the finding that the B_{12} malabsorption patients had not lost significantly more weight than the patients with normal B_{12} absorption (Table I).

The D-xylose test was abnormal in most patients, which is in agreement with Berchtold's findings but contrary to the findings of Willms and Creutzfeldt (1970). However, the importance of abnormal D-xylose tests in our patients is doubtful as D-xylose excretion has been shown to be decreased in normal subjects over the age of 65 and the mean age of our patients was 63·3 years (S.E. of mean 1·95) (Fowler and Cooke, 1960).

It is concluded that malabsorption of B_{12} in patients on long-term metformin therapy is a frequent finding and of clinical importance as 21 of the patients examined had B_{12} malabsorption and four of these were found within six years of beginning therapy to be B_{12} deficient. This drug was introduced to general use only about 10 years ago and it is to be expected that further patients with B_{12} deficiency will be encountered as more patients continue to take the drug for longer periods.

We think that it is important that all patients on long-term metformin therapy should have annual serum B_{12} estimations, as there is a risk that the initial symptoms of subacute combined degeneration of the cord may be mistaken for diabetic neuropathy in these patients.

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