There is a relation between the development of the disease in susceptible men and working in coal seams of intermediate height—3 ft. to 4 ft. 11 in. (91 to 150 cm.).

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TREATMENT OF PERNICIOUS ANAEMIA WITH ORAL VITAMIN B₁₂ WITHOUT KNOWN SOURCE OF INTRINSIC FACTOR

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Several groups of workers have investigated the effect of orally administered vitamin B₁₂ in the megaloblastic anaemias. The majority of the reports have come from America, and include those by Berk *et al.* (1948), Spies *et al.* (1949), Hall (1950), Meyer *et al.* (1950), Conley *et al.* (1951, 1952), Glass *et al.* (1952, 1953), and Unglaub *et al.* (1954). British publications are those of Ungley (1950, 1951-2), and Girdwood (1953).

It appears from these reports that single oral doses of vitamin B₁₂ of less than 1,000 µg. have, in general, little effect, and that, while repeated daily doses of 50-100 µg. may cause some haematological response, the degree and length of improvement have been very variable. In some instances no responses were obtained. Spies and his colleagues concluded that a daily oral dose some 30 to 60 times that of a single intramuscular injection is necessary to obtain similar effect.

Ungley (1950) first showed that a single massive oral dose of 3,000 µg. of vitamin B₁₂ without any intrinsic factor was therapeutically effective in cases of pernicious anaemia

in relapse, and this was confirmed by Conley *et al.* (1951) and Chalmers *et al.* (1951). The haemopoietic effect of smaller doses of oral vitamin B₁₂ has been shown to be enhanced by giving it with normal gastric juice and other sources of intrinsic factor (see Ungley, 1950; Glass and Boyd, 1953; Latner *et al.*, 1953). Haematological responses have also been reported in cases of pernicious anaemia treated with oral aureomycin (Lichtman *et al.*, 1950), and oral and parenteral penicillin were found to be effective in the megaloblastic anaemias described by Foy *et al.* (1952). If the intrinsic factor present in normal gastric juice acts by protecting vitamin B₁₂ from utilization by intestinal bacteria, as was indirectly suggested by the work of Ternberg and Eakin (1949), these antibiotics may have increased absorption of the vitamin by alteration of the bacterial flora. Ungley (1950) tried to prove this by introducing vitamin B₁₂ into a washed portion and a partially sterilized portion of the intestines of patients with pernicious anaemia in relapse, but no responses were obtained.

In Ungley's investigations the patients were given oral doses of vitamin B₁₂ when fasting, and this condition was strictly observed in the following experiments, in which oral vitamin B₁₂, in amounts ranging from 9,000 µg. in a single dose to repeated daily doses of 50 µg., was administered without known source of intrinsic factor to cases of megaloblastic anaemia in relapse.

Material and Methods

Twelve of the patients studied were suffering from pernicious anaemia in relapse, with typical blood values, megaloblastic marrows, and histamine-fast achlorhydria. Three others had undergone gastrectomy, but resembled cases of pernicious anaemia in other respects, including their ultimate complete response to treatment with vitamin B₁₂. No free hydrochloric acid was found when test meals were attempted on these patients, and there were no symptoms or signs of steatorrhoea. Fat-balance studies were not made. Oral vitamin B₁₂ was given after different periods of observation to ensure that there was a maintained fall in pre-treatment blood-count values; Case 11, who was known to have suffered from pernicious anaemia previously and to have responded to parenteral liver extract, did not want treatment restarted "until it was necessary," and provided for us a prolonged period of control observation.

When in hospital, the patients were placed on a diet from which offal was excluded, with restriction of eggs, meat, milk, and cheese. Patients receiving single amounts of oral vitamin B₁₂ were given the dose when fasting at 9 a.m., and no food or drink was permitted for four hours afterwards. When daily doses were employed it was found convenient to give the vitamin at midnight, no food or drink being taken between the hours of 6 p.m. and 6 a.m.

TABLE I.—Cases of Pernicious Anaemia and Post-gastrectomy Megaloblastic Anaemia treated with Single Oral Doses of Vitamin B₁₂

Case	Age and Sex	Single Dose Oral Vitamin B ₁₂	Initial Blood Values (Pre-Treatment)			Values on 15th Day after Single Dose of Oral Vitamin B ₁₂			Reticulocyte Response			Marrow after Treatment. Day of Test in Parentheses
			Hb G. %	R.B.C. Mills. per c.mm.	P.C.V. %	Hb G. %	R.B.C. Mills. per c.mm.	P.C.V. %	Max. %	Day of Maximum	Type of Response	
1	53 F	9,000 µg.	9.9	2.30	29	12.7	3.44	40	11.4	5	Sharp	—
2	70 F	6,000 "	5.2	1.14	15	8.9	2.80	31	22.5	5		—
3	38 M	3,000 "	11.1	2.35	30	13.9	3.80	40	13.0	5		—
4	65 M	3,000 "	8.1	1.95	23.5	10.7	2.80	32	32.0	5		Normoblastic (6)
5*	56 F	3,000 "	7.4	1.55	23	11.1	2.95	38	32.0	6		Marked increase of normoblasts. Megaloblasts disappearing (2)
6*	40 M	2,000 "	11.5	2.80	34	13.3	3.70	39.5	9.5	5	Low flat	Some increase of normoblasts, but still many megaloblasts (3)
7*	47 M	1,000 "	6.8	1.42	19	7.1	1.50 (9th day)	21	8.6	6		

* Megaloblastic anaemia occurring in patients who had undergone gastrectomy.

TABLE II.—Cases of Pernicious Anaemia Treated with Daily Doses of 50 μ g. Oral Vitamin B₁₂

Daily Dose of Oral Vitamin B ₁₂	Case	Age and Sex	Initial Blood Values (Pre-Treatment)			Values on 15th Day of Oral Vitamin B ₁₂ Therapy			Reticulocyte Response			Marrow after Treatment. Day of Test in Parentheses
			Hb G. %	R.B.C. Mills. per c.mm.	P.C.V. %	Hb G. %	R.B.C. Mills. per c.mm.	P.C.V. %	Max. %	Day of Maximum	Type of Response	
50 μ g. with 0.5 g. "tween twenty" when fasting	8	59 F	6.2	1.80	18.5	9.5	2.48	28	29.0	9	Usually prolonged and of "plateau" type	Normoblastic with some transitional forms (11)
	9	44 M	9.2	2.10	26	11.8	3.02	33	13.0	10		Mainly normoblastic, but transitional forms present (11)
50 μ g. in saline when fasting	10	53 F	5.9	1.50	17	8.6	2.20	27	17.0	8		Normoblastic (12)
	11	58 F	10.4	2.60	33	12.3	4.20	39	8.0	9		" (12)
	12	67 F	12.1	2.36	33	13.2	3.60	36.5	6.2	8		" (15)
	13	62 F	8.0	1.80	25	10.2	2.66	31	9.5	8		" (8)
	14	70 F	4.9	0.97	14	7.7	1.82	23	15.6	9		—
50 μ g. in saline after midday meal	15	72 M	10.4	2.34	31	11.8	2.60	33	7.3	10		Normoblast increase. Still some megaloblasts and transitional forms (15)

Technical methods were the same as described previously (Chalmers, 1951), except that a photoelectric colorimeter with Thompson's grey standard, calibrated so that a recording of 100% was equivalent to 14.8 g. Hb per 100 ml., was used throughout.

Results

Effect of Large Single Doses of Oral Vitamin B₁₂

Haematological responses of Cases 1 to 7 are shown in Table I. Single doses of oral vitamin B₁₂ in saline given to the fasting patients resulted in prompt haematological improvement in six of them when the dose administered was 2,000 μ g. or more. Sharp reticulocyte peaks on the fifth or sixth day compared well with the results obtained by us with single intramuscular doses of 20–100 μ g. of the vitamin. With the exception of Cases 4 and 7, all satisfied the formula of Della Vida and Dyke (1942) in their average weekly red-cell gain.

While Case 4 almost reached this arbitrary standard, Case 7 showed only a slight increase in red cells and reticulocytes after an oral dose of 1,000 μ g., and subsequently had a secondary reticulocyte response of 26.5% after a single intramuscular

FIG. 2.—Case 11. Results of treatment with oral vitamin B₁₂ alone over a period of 18 months.

injection of 200 μ g. vitamin B₁₂ given on the tenth day of the experiment.

FIG. 1.—Case 11. Initial response following oral vitamin B₁₂ therapy.

Effect of Smaller Daily Doses of Oral Vitamin B₁₂

At the suggestion of Dr. W. F. J. Cuthbertson two patients suffering from pernicious anaemia in relapse were treated with oral vitamin B₁₂ in daily doses of 50 μ g. combined with 0.2 g. of the surface agent polyoxyethylene sorbitan monolaurate ("tween twenty"), to see if this substance could act as an aid to absorption of the vitamin. No food or drink was taken for six hours before or after the dose was given, and the experiment was continued for 14 days. Significant clinical and haematological improvement occurred in both cases (Table II, Cases 8 and 9). Reticulocyte responses usually of a prolonged or "plateau" type were observed, the maximum levels being on the ninth and tenth days after the starting of treatment. The marrows became essentially normoblastic by the eleventh day.

Control experiments were then carried out on five further cases of pernicious anaemia in relapse, giving 50 μ g. of oral vitamin B₁₂ without the surface agent for 14 days under the same test conditions. Equally good clinical and haematological improvement was obtained.

In one patient (Case 15) the same dose of the vitamin taken immediately after the midday meal showed much less clinical effect, although a small increase in red cells, reticulocytes, and packed cell volume occurred, together with some increase of normoblasts in the marrow.

Though facilities did not exist for following up most of the cases, it was possible to arrange for Case 11, who

had already accommodated us with a very adequate control period before treatment, to be maintained on doses of 50 μ g. of oral vitamin B₁₂. Details of this case, showing haematological values for over a period of two and a half years, are shown in Figs. 1 and 2. She had a primary response that satisfied Della Vida's formula and has kept in haematological remission for 18 months, during which time she has received no other treatment than 50 μ g. of oral vitamin B₁₂ taken every night before retiring.

Discussion

The experiments show that, under the conditions of the clinical trials described, oral vitamin B₁₂ without added source of intrinsic factor is haemopoietically active when given either in large single doses of 2,000 μ g. or more or in repeated daily doses of 50 μ g. The results of the experiments in which the vitamin was given in saline do not suggest that tween twenty increased absorption of the vitamin to any significant extent. The uniformly good results obtained with oral daily doses of 50 μ g. could be related to the strict precautions taken to ensure that the patients took the dose when fasting and had no food for six hours afterwards.

There are several possible reasons for this. (1) In the fasting state there is greater opportunity for the vitamin to come into contact with the intestinal mucosa than when it is mixed with food. Close contact between the intestinal mucosa and vitamin B₁₂ in adequate concentration may be the necessary condition for the absorption of the vitamin without intrinsic factor. Castle and his colleagues have now suggested that the role of intrinsic factor is merely to increase assimilation of vitamin B₁₂, and that its effect may be on the intestinal wall rather than directly on the vitamin (see Berk *et al.*, 1948; Wallerstein *et al.*, 1953). (2) Vitamin B₁₂ may be destroyed by digestive secretions unless protected by intrinsic factor (Hall, 1950) and, if so, the small amount of secretions in the fasting state may not destroy all of the 50 μ g. dose. (3) If intrinsic factor prevents utilization of vitamin B₁₂ by bacteria, some temporary alteration in bacterial flora when the patient is fasting may allow the vitamin to be absorbed.

Another possible reason for the responses obtained could be that the patients with histamine-fast achlorhydria were still secreting a small amount of intrinsic factor. It has been suggested that lack of intrinsic factor is not absolute in all cases of pernicious anaemia (Goldhamer, 1936; Campbell *et al.*, 1949), and, while it might seem unlikely that we would encounter this condition in a series of consecutive cases, the possibility cannot be excluded. To prove that the fasting state was important in allowing absorption of the vitamin, it would be necessary to use each patient as his own "control," giving the vitamin first with food and, later, in similar dosage when fasting. It was unfortunately not possible to carry out such studies on the present series of cases or to draw conclusions when smaller doses were given under the same conditions.

While the reason is not yet understood, it would seem that oral vitamin B₁₂—now accepted as "extrinsic factor"—can cause haematological response in cases of pernicious anaemia in the absence of any known source of intrinsic factor. We do not advocate this method of oral therapy for routine management of cases of pernicious anaemia, but our results demonstrate again that extraneous sources of intrinsic factor are not essential for utilization of vitamin B₁₂ and that oral therapy with the vitamin alone may be most effective when given to fasting patients.

Summary

Cases of megaloblastic anaemia in relapse given single oral doses of 2,000–9,000 μ g. of vitamin B₁₂ showed initial haematological responses similar to those obtained by a single intramuscular injection of 20–100 μ g. of the vitamin.

Daily doses of 50 μ g. of oral vitamin B₁₂ taken when fasting caused significant haematological responses in cases of pernicious anaemia in relapse. A less satisfactory response was obtained when the same dose was taken with food.

The surface agent "tween twenty" did not appear to be of value in aiding absorption of vitamin B₁₂.

A case of pernicious anaemia in relapse has been initially treated and maintained in remission for over 18 months on a dose of 50 μ g. of oral vitamin B₁₂ taken daily on retiring at night.

While intramuscular preparations are preferable and more convenient for routine management, it is concluded that, without intrinsic factor, oral vitamin B₁₂ can be utilized when taken fasting by patients suffering from pernicious anaemia and that clinical and haematological remission can be obtained with oral vitamin B₁₂ alone.

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Pharmaceutical exports declined slightly in 1953 to £30.7m. from £31.7m. in 1952. According to the *Annual Report* for 1953–4 of the Association of British Pharmaceutical Industry the main reasons were the fall in world prices and the export of bulk instead of finished goods. The principal market was India, which took £3m. worth; British West Africa and Australia came next with over £2m. Exports to the Union of South Africa, New Zealand, Malaya, Hong Kong, and Eire each exceeded £1m. Difficulties were experienced in trade with Pakistan and Egypt. In 1952 Pakistan was Britain's second best customer with £2m., but, owing to the reduction in her own exports, imports of drugs were curtailed and fell to £823,000. Exports to Brazil, formerly about £500,000, almost entirely disappeared. Countries poorly developed industrially are beginning to manufacture drugs or at least finish the later stages. Consequently material is being exported in bulk for local finishing and profits are lower. The Chinese government is said to be embarking on a manufacturing programme which will make it independent of all outside supplies of drugs.