LONG-TERM TRIAL OF ORAL TREATMENT OF PERNICIOUS ANAEMIA WITH VITAMIN-B₁₂-PEPTIDE

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When it was suggested that pernicious anaemia could be successfully treated by an oral preparation of vitamin B₁₂ as a peptide complex (Heathcote and Mooney, 1958) it was decided to treat a group of patients suffering from pernicious anaemia with this preparation. We decided to regard a response as satisfactory if the treatment was able to maintain not only the haemoglobin level but also the serum-vitamin-B₁₂ concentration at a satisfactory level.

Material and Methods

Twelve untreated patients (six men, six women) were chosen. All satisfied the clinical and haematological criteria for the diagnosis of pernicious anaemia—namely, macrocytic anaemia, megaloblastic bone-marrow, histamine-free achlorhydria, and a low serum vitamin B₁₂.

The following data were recorded in each instance: haemoglobin concentration, red-cell count, packed cell volume, maximum reticulocyte response, together with the day on which this was obtained, and serum vitamin B₁₂. The haemoglobin was estimated by the alkaline haematin method using a photoelectric colorimeter; the red-cell counts and packed cell volume were measured by standard methods, and the serum vitamin B₁₂ by means of *Euglena gracilis* (Ross, 1952), using "Z" strain (Hutner *et al.*, 1956).

Each patient was given the peptide preparation and followed up for periods ranging from 34 to 49 months. Vitamin-B₁₂-peptide 100 µg. three times daily was given for 14 days and then reduced to 100 µg. daily. It was planned to lower the dose if an adequate serum-vitamin-B₁₂ level could be maintained; 250 µg./ml. was regarded as satisfactory, as this was the mean figure obtained from assaying sera from two random samples of the population (to be published).

Results

Table I gives the age and the sex of the patients, the initial P.C.V., the maximum reticulocyte response, and the day on which it occurred. All the cases except number 7, about which full information is lacking, had

TABLE I.—Initial Response to Therapy with Oral Vitamin-B₁₂-Peptide

Case No.	Age	Sex	Initial Serum Vitamin B ₁₂ (µg. ml.)	Initial P.C.V. (%)	Maximum Retic. (%)	Day
1	59	F	50	19	22.2	12
2	65	M	8	22	13.8	9
3	68	F	21	20	28.0	7
4	44	M	7	32	13.0	9
5	72	M	11	15	24.6	10
6	67	F	18	30	15.6	7
7	67	F	25	34	—	—
8	67	M	12	14	24.8	6
9	50	M	82	15	18.2	10
10	65	F	47	22	20.4	7
11	71	F	9	27	23.0	8
12	72	M	20	27	19.2	8

a reticulocyte response which compares favourably with that obtained by conventional therapy. The haemoglobin, R.B.C., and P.C.V. continued to rise, and within two months (Table II) from the beginning of treatment had risen to normal levels in all except one (Case 9) who about this time developed a liver dysfunction with an increase in serum gamma-globulin, giving reversal of the albumin/globulin ratio together with an iron-

TABLE II.—Haematological Values After Two Months' Oral Therapy

Case No.	Hb (g./100 ml.)	R.B.C. (millions/c.mm.)	P.C.V. (%)	Serum Vitamin B ₁₂ (µg./ml.)
1	14.4	5.3	43	276
2	16.1	5.0	46	164
3	14.1	4.5	44	147
4	16.8	5.6	50	168
5	13.0	4.5	42	91
6	13.8	4.5	43	143
7	14.5	4.7	40	120
8	15.7	5.5	48	178
9	8.9	3.5	30	157
10	13.3	4.4	44	252
11	13.5	—	46	233
12	14.7	4.6	45	265

deficiency anaemia. However, four months later, when the serum proteins had returned to normal and the iron deficiency had been corrected, his haemoglobin reached 15.7 g./100 ml. Table III shows the serum-vitamin-B₁₂ levels, and the dosage of vitamin-B₁₂-peptide complex ("distivit") at monthly intervals for the first seven months and at three-monthly intervals. The clinical condition and the haemoglobin levels of all the patients have remained satisfactory throughout the period of observation.

As treatment continued it was found that Cases 2, 3, 5, 6, 7, and 8 (Table III) were not maintaining a satisfactory serum level of vitamin B₁₂. The dosage of the peptide complex was therefore increased from 100 µg. daily to 300 µg. daily, when in each case the serum vitamin B₁₂ rose to an adequate level and has remained there. One patient (Case 4), a man with symptoms and signs of subacute combined degeneration of the cord, was kept on a daily dose of 300 µg. throughout. The neurological lesions improved, leaving no disability.

Case 10 has been maintained on 200 µg. daily for a period of 40 months with an adequate serum level of vitamin B₁₂. Case 1 was kept on 100 µg. daily for 16 months quite satisfactorily, but the dosage was then increased to 300 µg. daily as in the others. Cases 11 and 12, because of our previous experience, were kept on the initial dosage of 300 µg. daily from the beginning of treatment.

Discussion

It was reported by Heathcote and Mooney (1958) that patients with pernicious anaemia treated with vitamin-B₁₂-peptide complex at the very low dosage of 10 µg./day remained clinically and haematologically satisfactory, though the serum-vitamin-B₁₂ levels were not

TABLE III.—Serum-vitamin-B₁₂ Levels in $\mu\text{g.}/\text{ml.}$ with Daily Oral Doses of Vitamin-B₁₂-Peptide Complex in $\mu\text{g.}$

Case No.		Months																				
		1	2	3	4	5	6	7	10	13	16	19	22	25	28	31	34	37	40	43	46	49
1	Serum B ₁₂	—	276	221	236	—	277	—	218	—	167	249	162	—	—	—	—	—	—	—	—	382
	Dose	100	1 300																			
2	Serum B ₁₂	165	164	102	—	116	—	94	158	—	179	—	—	170	—	228	—	240	—	195		
	Dose	100	1 300																			
3	Serum B ₁₂	221	147	119	95	—	139	286	—	—	210	—	272	—	247	—	274					
	Dose	100	1 300																			
4	Serum B ₁₂	187	168	177	—	153	194	—	—	—	—	—	—	258	—	—	213	—	—	—	300	
	Dose	300																				
5	Serum B ₁₂	114	91	119	152	138	210	236	—	172	141	213	—	235	228	223	—	297	—	311	—	280
	Dose	100	1 300																			
6	Serum B ₁₂	112	143	90	—	185	221	280	254	217	—	319	322	—	—	—	274	—	—	388		
	Dose	100	1 300																			
7	Serum B ₁₂	138	120	138	157	273	184	—	354	—	354	550	520	—	—	—	—	576				
	Dose	100	300																			
8	Serum B ₁₂	—	178	137	112	127	196	—	286	204	—	260	292	—	—	—	—	290				
	Dose	100	1 300																			
9	Serum B ₁₂	—	157	239	310	412	347	—	224	—	226	—	—	—	—	—	—	—	229			
	Dose	100	1 300																			
10	Serum B ₁₂	155	252	228	—	—	—	—	225	—	—	230	—	343	—	—	—	—	317			
	Dose	200																				
11	Serum B ₁₂	254	177	233	307	—	—	300	299	—	—	—	311	—	—	—	206					
	Dose	300																				
12	Serum B ₁₂	188	265	294	218	—	—	—	242	—	299	279	—	—	—	392	—	—	342			
	Dose	300																				

determined. We found that an adequate level of vitamin B₁₂ was not maintained with the much higher dose of 100 $\mu\text{g.}$ daily, thus confirming the finding of Varadi and Elwis (1960), who gave 100 $\mu\text{g.}$ t.d.s. for 10 days followed by 100 $\mu\text{g.}$ daily. In two of their patients the serum-vitamin-B₁₂ levels remained below normal during the treatment and in three it decreased after an initial rise. Shinton (1961) carried out a careful trial of the peptide complex of vitamin B₁₂, and while he demonstrated a rapid improvement in all haematological indices, only those patients who were having 500 $\mu\text{g.}$ daily obtained serum levels approaching his established mean level of about 400 $\mu\text{g.}/\text{ml.}$ Those patients on 200 $\mu\text{g.}$ of peptide daily did not have values rising above 200 $\mu\text{g.}/\text{ml.}$

Milhaud (1961) has shown that the vitamin-B₁₂-peptide complex is absorbed to a greater extent than crystalline vitamin B₁₂ in patients who have had a partial gastrectomy, and Heathcote and Mooney (1958) have shown that the peptide complex will induce maturation of megaloblasts *in vitro*. A reticulocyte response may also occur when very small quantities of vitamin-B₁₂-peptide are given (Mooney and Heathcote, 1961). Despite these possible theoretical advantages for treatment, however, we and others find that in patients suffering from pernicious anaemia small amounts of vitamin-B₁₂-peptide complex do not maintain the serum-vitamin-B₁₂ concentration at a satisfactory level.

Previous reports on crystalline vitamin-B₁₂ oral therapy (Reisner *et al.*, 1955; Conley and Krevans, 1955; Brody *et al.*, 1959; Thompson *et al.*, 1962) show that doses ranging from 100 $\mu\text{g.}$ daily to 1,000 $\mu\text{g.}$

weekly produce rapid and complete haematological remission, but failed to maintain the serum-vitamin-B₁₂ level satisfactorily. Only Chalmers and Shinton (1958) have been able to maintain satisfactory vitamin-B₁₂ levels on 100 $\mu\text{g.}$ daily.

It is considered that any oral therapy for pernicious anaemia must be given in a dosage sufficient to maintain adequate serum-vitamin-B₁₂ concentrations. It is well recognized that estimation of the haemoglobin alone is insufficient, and it is recommended that the serum level of vitamin B₁₂ be estimated at intervals in any patient having oral therapy.

In all our patients serum-vitamin-B₁₂ levels (Table III) have been maintained for periods varying between 34 and 49 months close to the mean normal level previously established. Our patients have also shown satisfactory progress both haematologically and neurologically, including the one patient (Case 4) who first presented with subacute combined degeneration of the cord, who showed marked improvement on therapy, and who has remained without further symptoms.

Although it is still our current practice to treat pernicious anaemia with parenteral vitamin B₁₂, we believe that the vitamin-B₁₂-peptide complex is a satisfactory method, provided an adequate dose—that is, 300 $\mu\text{g.}$ daily—is given. Because of the dangers of failure to take the required treatment and the known reluctance of patients to continue medication over long periods of time it is suggested that oral therapy might be indicated for those patients who could be relied on to take the treatment regularly and those who find repeated injections difficult to accept.

Summary

Twelve patients with Addisonian pernicious anaemia were treated with daily oral doses of vitamin-B₁₂-peptide complex for up to four years. While a dose of 100 µg. of the peptide complex daily was sufficient to maintain normal haemoglobin values it was insufficient to maintain the serum-vitamin-B₁₂ level.

A daily dose of 300 µg. of the peptide complex, however, was adequate, and it is suggested that such a method of therapy is safe and is of particular value in people who dislike injections.

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ORAL TREATMENT OF SUBACUTE COMBINED DEGENERATION OF SPINAL CORD

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From time to time during the past four years we have reported results obtained from the oral treatment of pernicious anaemia, using vitamin-B₁₂-peptide complexes (Heathcote and Mooney, 1958a, 1958b, 1961; Mooney and Heathcote, 1960, 1961a). With the exception of one patient mentioned in our first communication, all were uncomplicated examples of the disease. The present paper describes the results in 11 cases that showed, in addition to pernicious anaemia, evidence of degeneration in the nervous system.

The theory upon which the treatment is based has been fully described in our previous communications. It rests mainly upon our belief that pernicious anaemia is due to impaired gastric proteolysis of bound forms of vitamin B₁₂, and that intrinsic factor—as currently understood—does not exist.

The diagnosis of megaloblastic anaemia was confirmed in each case by sternal-marrow examination, histamine-alcohol test meal, and by examination of the peripheral blood. Haematological progress was assessed by packed cell volume (P.C.V.) readings performed on the Hawksley microcentrifuge, and by haemoglobin estimation (100% Hb = 14.8 g./100 ml.).

The serum-vitamin-B₁₂ concentration was estimated by the acidimetric titration of 72-hour cultures of *Lactobacillus leichmanii* using "Difco" vitamin-B₁₂ assay broth. All sera were cyanide-treated before assay.

All except Case 7—which in the early stages of treatment received a vitamin-B₁₂-peptide derived from a streptomyces source—have been treated with complexes derived from a propionibacterium. The ratio of associated peptide has varied slightly in the different complexes but has never exceeded 7.1:1.

The doses of the different batches of the peptide complex are given in terms of the cobalamin content alone as estimated spectrophotometrically at 550 mµ. Where crystalline vitamin B₁₂ has been used as such it is mentioned specifically in the text.

Case 7

A woman aged 52 was treated for pernicious anaemia with one tablet of "bifacton" twice daily for 16 months.

At the end of that time, despite the achievement and maintenance of a satisfactory blood-picture, she noticed that her thighs and legs were becoming stiff and difficult to move. Numbness of the feet also appeared, but the hands were unaffected. Vibration sense was lost over the ankles; knee- and ankle-jerks were diminished, and the plantar responses were equivocal. The red-cell count was 4,300,000/c.mm. and the Hb 94% at this time. Early subacute combined degeneration of the spinal cord was diagnosed, and she was treated initially with a streptomyces complex. For three weeks she received 300 µg. daily, and thereafter 200 µg./day for eight weeks. Oral treatment was then suspended owing to shortage of experimental material, and parenteral crystalline vitamin B₁₂ (1,000 µg.) was given every week for a whole year. Oral treatment was then restarted, but with a propionibacterium vitamin-B₁₂-peptide complex at a daily dose of 300 µg. This has remained unchanged up to the time of writing (234th week).

During the first period of oral treatment the stiffness in the lower limbs lessened considerably and her walking improved. Numbness in the feet diminished until only the toes were affected. Vibration sense returned to the left ankle, and also to the right external malleolus during this period, but there was no detectable change in the deep reflexes. During parenteral therapy the improvement in walking continued at about the same rate, but no further change appeared in the objective signs.

The second period of oral treatment began on the 440th day, and by the 666th day vibration sense had returned to the remaining (right internal) malleolus. By this time the only residual symptom was a slight tingling in the feet which came on when she was tired. She returned to work as an office cleaner on the 600th day.

At the time of writing the Hb was 92% and P.C.V. 42%. Her serum-vitamin-B₁₂ level was 240 µµg./ml. The knee- and ankle-jerks were normal but the plantar responses remained equivocal.

Case 34

A man aged 60 had a haemoglobin of 69% and a P.C.V. of 27%. The serum vitamin B₁₂ was 30 µµg./ml. His presenting symptoms were weakness, asthenia, and coldness and numbness of the legs and pelvis, together with severe lower abdominal girdle-pains on defaecation. His knees and ankles were stiff and the leg reflexes were absent. Plantar responses were flexor, but vibration sense was absent from the knees downwards. The gait was extremely