Oral Treatment of Pernicious Anaemia: First Fifty Cases

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From time to time in the past we have reported the results obtained from the oral treatment of pernicious anaemia with vitamin-B₁₂-peptide complexes (Heathcote and Mooney, 1958a, 1958b, 1962; Mooney and Heathcote, 1960, 1961a, 1963). We have now reviewed the first 50 unselected patients, who have been on this form of treatment for periods ranging from one to seven years, and the findings form the basis of this communication. The theory upon which the treatment is based has been fully described in our previous communications.

The diagnosis of pernicious anaemia was confirmed in each case by sternal-marrow examination, by histamine-alcohol test meal, and by examination of the peripheral blood. Haematological progress was assessed by the 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*, Difco vitamin-B₁₂ assay broth being used; sera were cyanide-treated before assay. All cases were treated with research batches of a complex derived from the fermentation of either a streptomyces or a propionibacterium. Commercial preparations were not used in the treatment of any of the cases. The ratio of associated peptide to vitamin B₁₂ has varied slightly in the different complexes but has never exceeded 7.1 to 1.

The doses of the different batches of the peptide complexes are given in terms of cobalamin content alone, as estimated spectrophotometrically at 550 mμ.

The results are summarized in Table I. Thirty-two of the patients are females and 18 are males. Their ages range from 39 to 84 years (average 62). Eight patients have left the district and are now lost to survey (see Table I). All these were in complete health when last seen.

A further nine patients died during the period under review, but in no case was death associated with pernicious anaemia either directly or indirectly. The average age at the time of death was 73.

The average daily maintenance dose of all 50 cases was 19.5 μg. of vitamin B₁₂, and 39 (78%) of them have been kept in health throughout on the original maintenance dose; in the remaining 11 (22%) cases an increase was made to the initial dose. Ten (20%) cases in the series developed iron deficiency, three developed mild neuropathic symptoms, and one developed an associated folic-acid deficiency. Those patients who in any way required an alteration in treatment during the period of survey are described in greater detail.

Neuropathy

Three patients (Cases 9, 18, and 22) developed neuropathy.

Case 9.—On the 2,160th day of treatment the patient, a woman aged 63, noticed a gradual onset of weakness, and of pins-and-needles in the fingers. These symptoms were severe enough to produce difficulty in buttoning clothes, holding needles, etc. The daily maintenance dose of complex at the time was 10 μg: the Hb

was 86%, the P.C.V. 38%, and the serum vitamin-B₁₂ level 40 μg./ml. A marrow puncture at this time showed megaloblastic changes in approximately 40% of the red-cell series. No abnormal signs were detected. The daily dose was therefore increased to 40 μg. of peptide complex, and by the 2,260th day the symptoms had disappeared, the blood picture had improved slightly, and the marrow had reverted to normal. The serum vitamin-B₁₂ level, however, remained unchanged at 40 μg./ml.

Case 18.—When first seen the patient, a man aged 74, complained of paraesthesiae of the hands among other symptoms, but it was thought at the time that this could have been due simply to the anaemia. However, on a daily dose of 10 μg. of vitamin-B₁₂-peptide, although the blood picture improved quite quickly, the paraesthesiae persisted. The daily dosage was therefore increased to 30 μg. on the 200th day and to 50 μg. on the 260th day. The paraesthesiae then disappeared completely on this higher dosage, but about four years later the same symptoms slowly returned, and

TABLE I.—Uncomplicated Cases of Pernicious Anaemia Treated with Vitamin-B₁₂-Peptides

Case No.	Age and Sex	Duration of Treatment (Days)	Vitamin-B ₁₂ -Peptide (as μg. Vitamin B ₁₂)		Initial Blood Picture			Latest Blood Picture			
			Mean Daily Dose	Current Maintenance Dose	Hb (%)	P.C.V. (%)	M.C.H.C. (%)	Hb (%)	P.C.V. (%)	M.C.H.C. (%)	Serum Vitamin B ₁₂ μg./ml.
1	51 M	2,604	10.6	10	66	22	44	102	44	33	83
2	78 F	2,550	10.9	10	48	19	37	93	42	33	70
3	70 M	2,489	15.4	20	39	15	38	92	43	31	220
4	70 M	2,468	10.6	10	76	27	42	97	41	34	82
5*	44 M	1,156	10.4	10	53	19	42	114	53	29	49
6a	77 M	1,926	22.0	20	33	13	33	100	42	35	66
8	65 F	2,389	10.3	10	39	19	31	100	44	33	360
9	63 F	2,260	12.0	40	50	20	37	92	42	32	40
10†	75 F	2,134	29.4	20	45	19	35	95	41	34	90
12*	72 F	1,970	40.0	30	47	20	35	91	41	33	109
13*	77 F	1,544	11.4	10	37	16	34	90	42	32	98
15	67 F	2,250	34.0	40	50	19	39	86	40	32	98
16	39 F	1,980	11.5	20	63	—	—	88	38	34	70
17†	76 F	652	19.8	20	22	14	24	76	39	29	38
18	80 M	2,187	44.0	100	49	22	33	84	40	31	70
19†	77 M	1,071	10.0	10	62	28	31	80	39	31	76
20	44 F	2,229	20.0	20	52	25	31	89	42	30	90
22	84 F	2,237	28.0	100	38	16	35	99	40	37	90
23†	78 F	901	15.0	15	59	25	35	98	41	36	88
24†	66 F	646	76.4	80	52	22	35	90	46	29	88
25	61 F	1,853	6.1	6	66	30	32	106	42	36	70
26†	58 M	1,456	3.5	4	50	25	30	99	42	35	60
27	64 M	1,978	5.8	10	56	26	32	87	37	34	70
28	69 M	1,755	38.1	50	45	19	35	91	37	36	60
29†	75 F	1,574	14.2	10	30	14	35	90	40	33	68
30	70 M	1,710	17.4	20	56	22	37	98	41	35	60
31†	71 M	1,195	17.9	20	69	31	33	92	45	30	60
36	72 M	1,700	20.0	20	70	33	32	102	42	36	78
38*	70 F	779	4.5	4.5	29	14	31	87	43	30	156
43	65 F	1,579	41.2	30	38	15	36	95	43	33	42
44	57 F	1,581	20.1	10	48	21	34	89	35	36	96
45	39 F	1,422	12.2	10	57	26	32	100	44	33	95
51†	83 F	451	20.0	20	48	31	35	76	38	29	95
54	70 F	1,500	—	10	60	26	34	97	40	35	95
56*	84 M	591	26.6	40	41	21	29	95	42	34	20
60	72 F	1,346	14.7	10	46	20	34	97	40	35	184
65	53 F	1,047	10.3	10	34	15	34	102	41	36	20
66	75 F	1,162	20.0	20	41	18	34	81	41	30	80
69*	70 F	603	13.6	10	53	22	35	98	44	32.5	130
70	62 M	1,001	15.6	10	43	18	34	85	40	31.5	24
75*	70 F	546	25.0	20	34	14	36	95	42	34	50
81	78 M	920	29.3	15	29	15	29	94	42	34	50
82	73 F	403	13.8	10	70	30.5	34	95	43	33	24
83*	42 F	380	23.1	20	40	17	34	95	41	35	66
84	80 F	869	20.7	10	35	15	34	92	40	34	46
85	61 F	858	8.1	5	64	27	35	88	40	32	60
88	69 F	811	22.0	20	48	21	34	88	40	33	40
89	74 F	840	28.4	20	45	19	35	94	43	32	55
95	63 M	747	22.5	20	61	30	30	95	42	33	46
98	42 M	797	14.2	10	42	15	42	83	36	34	46
Average	62 —	1,397	19.5	19.2	49	20	35	92	41	33	82

* Patient left district during period of survey.

† Patient died during period of survey.

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this time were associated with a weakness of the hands which prevented the patient from gripping objects securely. Co-ordinated movements of the hands—for example, shaving—were also affected. The blood picture, too, had begun to deteriorate (Hb 64%, P.C.V. 27%, M.C.H.C. 34%). On the 1,820th day the daily dosage was increased to 100 μ g. of vitamin-B₁₂-peptide. This brought about a rapid improvement in the blood picture; the neurological symptoms, too, quickly disappeared and have not recurred since (2,187th day). At all levels of treatment the serum vitamin-B₁₂ concentration has never exceeded 80 μ g./ml., and the latest reading, also taken on the 2,187th day, was 38 μ g./ml.

Case 22.—A woman aged 84 had been satisfactorily maintained on a daily dosage of 10 μ g. of vitamin-B₁₂-peptide for almost five years when she began to complain of an increasing stiffness in the lower limbs and of difficulty in walking. Examination revealed no abnormality beyond absent ankle-jerks—a discovery of limited value at her age—and a marked osteoarthritis of the knees. The blood picture at that time was Hb 99%, P.C.V. 40%. The serum vitamin-B₁₂ level was 66 μ g./ml. Owing to circumstances, it was not possible to confirm a diagnosis of neuropathy by marrow puncture, and it was uncertain at first whether the symptoms were not entirely due to osteoarthritis. However, when the dosage of vitamin-B₁₂-peptide was increased to 100 μ g./day a marked improvement in her lower limbs took place and walking became considerably easier. Despite the marked increase in the daily dosage of the complex, the vitamin-B₁₂ concentration remained low and was only 76 μ g./ml. on the 2,237th day.

Increase in Maintenance Dose

Apart from the increases required in the three neurological cases mentioned above, it became necessary also to augment the initial maintenance dosage of a further eight cases (Table II).

TABLE II

Case No.	Day of Increase	Increase (μ g. B ₁₂ Daily)	
		From	To
3	1,873	10	20
15	1,296	30	40
16	1,796	10	20
24	175	10	80
27	1,707	4	10
28	1,120	30	50
30	481	10	20
56	491	20	40

With the exception of Case 24, a small increase in the maintenance dose was sufficient to correct the blood picture. Furthermore, these adjustments became necessary only after prolonged treatment (average period, 42 months), and there can be little doubt that they were due to a slow spontaneous deterioration in the disease itself. In this connexion Blackburn *et al.* (1952) also observed a similar deterioration in patients after prolonged parenteral vitamin-B₁₂ therapy.

Case 24, however, appears to be a case of partial refractoriness, because a disproportionately large increase in the daily maintenance dose became necessary after only six months' treatment. This case is discussed below.

Iron Deficiency

Ten cases either had iron deficiency at the time of diagnosis of pernicious anaemia or developed it subsequently to a degree severe enough to require treatment. Details are given in Table III.

In Cases 17 and 81 iron deficiency was present at the time of diagnosis of pernicious anaemia, and in Cases 29 and 45 it became apparent shortly after the beginning of treatment. One patient (Case 85) had suffered from iron-deficiency anaemia for 11 years prior to the onset of pernicious anaemia, but this was latent at the actual beginning of vitamin-B₁₂-peptide therapy. In the remaining cases iron deficiency became apparent after

periods ranging from five months (Case 84) to four years (Case 6a) from the beginning of treatment for pernicious anaemia.

TABLE III

Case No.	Iron Therapy Begun (Days)	Blood Picture at Beginning of Iron Therapy		
		Hb %	P.C.V. %	M.C.H.C. %
6a	1,565	77	36	32
17	1	22	14	24
20	294	71	35	29
29	48	36	18	29
44	785	63	35	26
45	22	63	33	28
81	6	33	18	27
84	147	60	38	24
85	1	64	27	35
95	308	66	33	29

Folic-acid Deficiency

In one case a relapse occurred which could be corrected only by the addition of folic acid to the treatment.

Case 54.—A woman aged 70 was treated from the 1st to the 181st day on 60 μ g. of vitamin-B₁₂-peptide, but during this period the improvement obtained was suboptimal and the highest recorded blood level was Hb 80%, P.C.V. 34%. On the 181st day the daily dosage was increased to 300 μ g. because the blood picture had begun to deteriorate, but, despite this, on the 209th day the Hb had fallen to 45% and the P.C.V. to 19%. Parenteral vitamin B₁₂ (1,000 μ g.) was therefore given, but three days later the Hb had dropped further to 42% and the P.C.V. to 18%, and no reticulocyte response was detectable. From the 212th day onwards 45 mg. of folic acid was given daily by mouth, in addition to 300 μ g. of vitamin-B₁₂-peptide. A brisk reticulocyte response resulted (maximum 22% on 217th day), and on the 219th day the haemoglobin had risen to 50% and the P.C.V. to 25%. The dosage of folic acid and vitamin-B₁₂-peptide was then gradually reduced until, by the 279th day, the daily maintenance dosage was 5 mg. of folic acid and 10 μ g. of vitamin-B₁₂-peptide. This dosage has remained unchanged up to the present (1,500th day). Currently the Hb is 97%, the P.C.V. 40%, and serum vitamin-B₁₂ level 96 μ g./ml.

Discussion

Serum Vitamin-B₁₂ Levels

The efficacy of our preparations has in the past been challenged because the serum vitamin-B₁₂ levels have tended to remain lower than normal on this form of therapy, when the patient has received daily doses of less than 100 μ g. (equivalent) of vitamin B₁₂ (Varadi, 1959; Varadi and Elwis, 1960; Shinton, 1960; Smith, 1961). These criticisms were largely based on the widespread but unproved assumption that serum vitamin-B₁₂ levels reflect the degree of tissue saturation on vitamin-B₁₂ therapy under all circumstances. Indeed, one of the critics (Varadi, 1959, 1960) actually classified a case as "relapsed" merely because the serum vitamin-B₁₂ level fell when the daily dosage was reduced from 300 to 100 μ g. of vitamin-B₁₂-peptide, though the blood picture did not deteriorate.

We have already shown, however, that if such an assumption were true it would also have to be conceded that the pernicious-anaemia patient is capable of absorbing several times more vitamin B₁₂ from the gut than does the normal average individual (Mooney and Heathcote, 1961b). Furthermore, vitamin B₁₂ is essentially an intracellular substance and is clearly not distributed in equal proportions between the body compartments. Thus, although the plasma makes up some 5% of the total body fluid, its own vitamin-B₁₂ content is less than 0.05% of the total amount found in the body. It would be more than optimistic, therefore, to assume that variations in this tiny amount in the plasma would necessarily, and in all circumstances, follow accurately the changes in the remaining 99.9% residing in the liver and other tissues.

Whatever the practical significance, if any, of the serum vitamin-B₁₂ levels on vitamin-B₁₂-peptide therapy, it is now clear from the evidence presented in Table I that low serum vitamin-B₁₂ levels are quite consistent with complete health for long periods on vitamin-B₁₂-peptide. Furthermore, in the three cases which developed neuropathy the onset was not preceded by a fall, nor was the clinical recovery accompanied by a significant rise, in the level of vitamin B₁₂ in the serum. In this connexion it is pertinent to recall the work of Banerjee and Chatterjea (1960), who have shown that low levels of serum vitamin B₁₂ are quite consistent with good health in normal individuals on natural low-protein diets.

Comparison with Crystalline Vitamin B₁₂

The effect of oral crystalline vitamin B₁₂ in pernicious anaemia has been studied extensively, and most of the work on this subject has been reviewed by Ungley (1952), by Brody *et al.* (1959), or by ourselves (Heathcote and Mooney, 1958a, 1958b). The differing schemes of dosage employed in the studies make it difficult to summarize the findings in precise terms, but there appears to be fairly general agreement that dosages of at least 100 µg. are required in most cases, and some patients may require more. In fact Britton (1963) states that, by mouth, vitamin B₁₂ is effective only when used in massive doses of 2,000 to 5,000 µg./day.

In our series, maintenance of the 50 cases is currently achieved within the following daily dose range: 10 µg. or less, 23 cases; 11 to 20 µg., 18 cases; 21 to 40 µg., 5 cases; 50 µg., 1 case; 80 µg., 1 case; 100 µg., 2 cases.

The two patients (Cases 18 and 22) who require a maintenance dose of 100 µg. daily are two of the three who had developed neuropathy on a lower dosage. The patient (Case 24) who required 80 µg. daily is the solitary example of partial refractoriness. These results, supported by the control experiments recorded in our earlier work (Mooney and Heathcote, 1960, 1961a), demonstrate that the vitamin-B₁₂-peptide complexes are much more effective than crystalline vitamin B₁₂ alone in the oral treatment of pernicious anaemia.

Refractoriness

This has been constantly observed in cases of pernicious anaemia treated with all so-called purified intrinsic-factor preparations (Meulengracht, 1953; Blackburn *et al.*, 1955; Nørregaard, 1955; Bastrup-Madsen, 1957; Schwartz *et al.*, 1957, 1958). Signs of refractoriness usually appear within 12 months and affect over half of all cases (Schwartz *et al.*, 1958). However, in our series of 50 patients, who have been treated exclusively with vitamin-B₁₂-peptide complexes for an average period of over three and a half years, only one case of partial refractoriness developed. This patient (Case 24) underwent a sudden deterioration after approximately six months' treatment, and this could be overcome only by increasing the dosage of vitamin-B₁₂-peptide from 10 to 80 µg. daily. On this higher dosage, however, a satisfactory blood picture was achieved and maintained until her death from cerebral haemorrhage over a year later.

Aetiology of Pernicious Anaemia

Although some 1,300 papers have now been written on "intrinsic factor" (for review see Glass, 1963), little is known about it except that it is alleged to be some form of protein which is capable of binding with vitamin B₁₂—a property shared by other proteins. We believe that the reason why intrinsic factor has not yet been isolated is simply that it does not exist.

Indeed, the theory upon which it depends (Castle, 1953) relies on two false assumptions.

The first assumption—that crystalline vitamin B₁₂ exists as such in animal protein—is no longer seriously defended, and for some years now it has been widely conceded that it is almost invariably attached to proteinaceous material in its natural form. The second assumption—that the large molecule of vitamin B₁₂ has to combine with another much larger molecule before it can be absorbed—defies all known principles of protein digestion.

In exact opposition to the view that vitamin B₁₂ has to be attached to a protein molecule (intrinsic factor) before absorption, we believe that the vitamin has to be detached to a greater or less degree from associated protein before absorption can take place. We believe that the essential step in this detachment is achieved by a specific gastric endopeptidase, deficiency of which results in the development of pernicious anaemia, and have suggested that this enzyme should be called "cobalamase" (Heathcote and Mooney, 1962).

In conclusion, we believe that vitamin-B₁₂-peptide complexes are effective in the oral treatment of pernicious anaemia simply because they resemble more closely the final form in which the vitamin is absorbed.

Summary

Fifty cases of uncomplicated pernicious anaemia have been treated with oral vitamin-B₁₂-peptides for periods ranging from one to seven years. The average daily dose was 19 µg. (vitamin-B₁₂ equivalent) and the average duration of treatment was approximately three and a half years.

Thirty-nine (78%) cases have been maintained throughout on the original maintenance dose of vitamin B₁₂.

Ten cases (20%) developed iron deficiency.

Three cases developed symptoms of mild neuropathy, which were corrected by increasing the dosage of vitamin-B₁₂-peptide.

One case developed a folic-acid deficiency and one case developed a partial resistance to therapy.

We believe that pernicious anaemia is caused by the lack of a specific gastric peptidase (cobalamase) which initiates the digestion of the protein at or about the site of attachment of the vitamin-B₁₂ molecule, and that the vitamin-B₁₂-peptide complexes are effective because they simulate to a greater, or less, degree the final form in which the vitamin is absorbed.

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