SUCCESSFUL MAINTENANCE OF PERNICIOUS ANAEMIA PATIENTS WITH VITAMIN B₁₂ AND INTRINSIC FACTOR FOR LONG PERIODS

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It is well established that many oral preparations of vitamin B₁₂ and hog intrinsic factor (I.F.) cause haematological and clinical remission in pernicious anaemia (P.A.). Recent reports have claimed that these oral preparations have failed to maintain a satisfactory remission in a variable percentage of the P.A. patients (Blackburn *et al.*, 1955; Adams, 1957; Killander, 1957, 1958a, 1958b; Kristensen *et al.*, 1957; Lowenstein *et al.*, 1957; Bastrup-Madsen, 1957; Schwartz *et al.*, 1957, 1958; Berlin *et al.*, 1958a, 1958b; Pedersen and Ebbesen, 1958; Schwartz, 1958; Stokes and Pitney, 1958). The inability of these preparations to maintain the patients was judged by haematological indices (vitamin B₁₂ serum levels, marrow examination, red blood cell count, and haemoglobin concentration) or by the measurement of the ability of the patients to absorb radioactive vitamin B₁₂ when given I.F. Most of these studies on refractoriness have been conducted with preparations manufactured and available in Europe under the trade marks "bendogen," "cycloplex," and "bifactor."

In the present report, an extension of a preliminary report (Lichtman *et al.*, 1958; Williams, 1959), 24 patients were maintained on various commercial intrinsic factor preparations for 5 to 67 months without significant development of impaired absorption of vitamin B₁₂.

Materials and Method

Two different commercial preparations from the American Cyanamid Company were used. They are designated as WES 374 and WES 592. Each capsule of WES 374 contained 50 mg. of hog I.F. and 30 µg. of vitamin B₁₂, which was equivalent to 2 U.S.P. units† and each capsule of WES 592 contained 60 mg. of I.F. and 15 µg. of vitamin B₁₂, which was equivalent to 1 U.S.P. unit, since WES 592 was less potent than WES 374. The patients were maintained on 1 capsule per day of the particular preparation studied.

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†The amount of vitamin B₁₂-I.F. preparation equivalent to 1 U.S.P. unit was determined by haematological assays in pernicious anaemia patients in relapse according to the requirements of the Anti-anemia Preparations Advisory Board of the U.S. Pharmacopoeia. The red-cell and reticulocyte responses were compared with the criteria established by the U.S.P. board. (The Fifth Announcement of the U.S.P. Anti-anemia Preparations Advisory Board, August 1, 1948, *J. Amer. pharm. Ass., pract. Pharm. Ed.*, 1948, 9, 660-669.)

The urinary excretion test as described by Ellenbogen *et al.* (1955) and Ellenbogen and Williams (1958) was used to determine quantitatively the ability of the patients to absorb vitamin B₁₂ with a standard I.F. preparation, 186-1. This preparation was previously studied by Baker and Mollin (1955), Callender and Evans (1955), Ellenbogen *et al.* (1955), Williams *et al.* (1956), and Ellenbogen and Williams (1958), and it was shown that 50 mg. of 186-1 was equivalent to the amount of intrinsic factor contained in 2-3 U.S.P. units.

All the urinary excretion tests in this report were conducted using 2 µg. (0.1-0.25 µc.) of vitamin B₁₂ labelled with ⁵⁶Co, ⁵⁸Co, or ⁶⁰Co. The radioactivity of the urine specimens was determined with a specially designed beaker of 1-litre capacity adapted to fit over and around a NaI well-type scintillation crystal (Ellenbogen and Williams, 1956) or by counting 200 ml. in a highly efficient plastic-well scintillator slightly larger than described (Hine and Miller, 1956).

Results

The results of the urinary excretion test of the 24 patients in the present study, prior to any oral therapy, is given in Table I. The average excretion without intrinsic factor was 0.9% and with 50 mg. 186-1 was 10.5%. This is in excellent agreement with the value of 0.7 and 10.5% respectively in 128 P.A. patients not maintained on oral therapy and not included in this study. Nine

TABLE I.—Urinary Excretion of Labelled Vitamin B₁₂ in Pernicious Anaemia Patients With and Without Standard Intrinsic Factor 186-1, Prior to Oral Therapy

Patient	Urinary Excretion Percentage of 2-µg. Oral Dose Excreted in 48 Hours	
	Without I.F.	With 50 mg. 186-1
1	0.9	9.9
2	0.4	—
3	0.1	12.9
4	0.5	19.2
5	0.6	4.2
6	1.0	6.1 (24-hour excretion)
7	1.5	15.3
8	1.6	12.1
9	1.0	3.9
10	2.3	17.5
11	—	—
12	0.3	16.2
13	1.1	6.6
14	0.4	10.5
15	0.5	8.8
16	1.4	13.8
17	0.2	—
18	0.5	7.5
19	0.3	6.8
20	0.1	5.7; 8.6
21	—	—
22	1.3	10.6
23	2.7	7.8
24	0	16.5
Averages	0.9	10.5

TABLE II.—Urinary Excretion Tests on P.A. Patients on Oral Maintenance (Intrinsic Factor Concentrate WES 374 plus Vitamin B₁₂)

Patients	Period on Previous Oral Maintenance	Period of This Study	Urinary Excretion (%) with WES 374 and 2 µg. Vitamin B ₁₂
1	14 months	11 months	11.9
2	4 "	17 "	12.4
3	8 "	14 "	7.7
4	10 "	18 "	10.1
5	16 "	12 "	15.2
6	15 "	7 "	9.9
7	9 "	15 "	14.4
8	36 "	15 "	19.5
9	12 "	18 "	7.3
Average			12.0

patients (Nos. 1-9) after a variable period of oral maintenance on various intrinsic factor preparations and/or the multihæmatinics containing intrinsic factor preparations were then started on maintenance for 7 to 18 months with I.F. preparation WES 374. After this period of oral therapy with WES 374 the urinary excretion test was performed on these nine patients with 100 mg. of I.F. WES 374. As can be seen from Table II, the patients all exhibited adequate excretion; the average excretion was 12.0%. No refractoriness was noted. The total period of oral therapy of these nine patients, taking into account the previous oral maintenance, varied from 21 to 51 months. Patient 8, who was maintained the longest on oral therapy, showed the best ability to absorb vitamin B₁₂ with I.F. WES 374.

At the completion of the study with WES 374 these same nine patients were placed on oral maintenance with I.F. WES 592 for 13 to 20 months. In five of these nine patients, immediately before the start of therapy with WES 592, the urinary excretion test was performed with WES 592. In addition, four to seven months after the start and at the completion of the period of study, the urinary excretion test was repeated. The data from this experiment are given in Table III. Except for Patient 6, there was no indication

TABLE III.—Urinary Excretion Tests on P.A. Patients on Oral Maintenance (Intrinsic Factor Concentrate WES 592 plus Vitamin B₁₂)

Patient	Period on Previous Oral Maintenance	Period of This Study	Urinary Excretion (%) with WES 592 and 2 µg. Vitamin B ₁₂		
			Start	During	End
1	25 months	17 months	6.1	4.8	6.0
2	21 "	13 "			9.2
3	22 "	20 "		4.0	3.5
4	28 "	17 "	10.5	14.1	7.8
5	28 "	15 "	12.0		9.4
6	22 "	17 "	9.6	9.0	3.8
7	24 "	18 "		10.5	9.2
8	51 "	16 "	17.6	13.9	12.2
9	30 "	16 "			3.2*
Averages			11.1	9.3	7.1

* Patient gave 3.9% with I.F.C. 186-1 on initial test prior to any oral therapy.

that the patients showed any significantly reduced ability to absorb vitamin B₁₂ with I.F. WES 592. Patient 9 had a limited ability to absorb vitamin B₁₂ with intrinsic factor prior to any oral therapy, and the inclusion of his excretion in the averages at the end of the test period as well as the reduced absorption exhibited by Patient 6 contributes to the lower overall average at the end of this study as compared with the average at the initiation of the study (7.1 versus 11.1%).

TABLE IV.—Urinary Excretion Tests on P.A. Patients on Oral Maintenance (Intrinsic Factor Concentrate WES 592 plus Vitamin B₁₂)

Patient	Period on Previous Oral Maintenance	Period of This Study	Urinary Excretion (%) with WES 592 and 2 µg. Vitamin B ₁₂		
			Start	During	End
10	0 months	19 months			8.8
11	34 "	17 "		12.4	9.4
12	0 "	14 "	11.6	15.5	11.7
13	30 "	14 "			10.3
14	0 "	15 "			16.2
15	34 "	17 "		8.3	6.2
16	0 "	5 "			9.2
17	32 "	19 "		10.2	5.2
18	0 "	5 "	10.1		12.9
19	0 "	14 "			7.3
20	0 "	15 "	8.0	6.1	5.0
21	17 "	13 "			8.7
22	0 "	7 "			8.0
23	28 "	17 "	12.5	13.9	12.8
24	0 "	11 "			10.0
Averages			10.5	11.0	9.7

Fourteen additional patients not previously maintained on other intrinsic factor preparations and/or the multihæmatinics containing intrinsic factor preparations were also placed on maintenance study with I.F. preparation WES 592 with urinary excretion tests performed at the start, four to seven months after, and at the completion of the maintenance period.

As can be seen from Table IV, the average excretion of these 14 patients at the end of the maintenance period did not differ significantly from that at the start of the maintenance period.

Discussion

The present investigation shows that development of resistance to intrinsic factor is not an inevitable occurrence of all hog intrinsic factor preparations after prolonged oral treatment in P.A. patients.

The failure of oral treatment has been attributed to the formation of antibodies to the hog proteins (Schwartz *et al.*, 1958). This is somewhat supported by the fact that vitamin B₁₂ absorption is inhibited in P.A. patients not previously treated orally when given vitamin B₁₂, I.F., and serum of patients previously treated orally with I.F. (Schwartz, 1958). Furthermore, many of these resistant patients absorb vitamin B₁₂ when it is given together with 50 ml. of normal human gastric juice. On the other hand, Berlin *et al.* (1958a, 1958b) have found that some P.A. patients who have developed a resistance to hog I.F. do not respond to 50 ml. of normal human gastric juice. No long-term studies have been conducted with P.A. patients to determine if they will develop resistance after maintenance with gastric juice for long periods. Intrinsic factor from the wild boar failed to affect the "blocked" absorption, whereas rat gastric juice was effective in re-establishing vitamin B₁₂ absorption (Schwartz *et al.*, 1958).

If the immunity theory is correct, this would imply that I.F. or some other protein component of the impure preparations is absorbed. As yet no data have been presented on whether I.F. is or is not absorbed. Furthermore, if one accepts the immunity theory as the cause of the blockage of absorption, then it must be assumed that during the fractionation and preparation of the cyanamid intrinsic factors used in this investigation the protein components giving rise to antibody formation were eliminated. Since some very crude preparations have been reported not to cause the development of resistance (Schwartz *et al.*, 1958), an alternative explanation may be that some unknown factor present in these crude preparations prevents the development of resistance. In view of the variable effects of different intrinsic factor preparations, Killander (1958a, 1958b) also believes that the development of resistance depends on the method of their manufacture.

Relatively large doses (the amount of intrinsic factor contained in the equivalent of 10 U.S.P. units or more) have been reported to abolish the "blocked" absorption in the resistant patients (Berlin *et al.*, 1958b). A patient not included in the maintenance study and who never received any oral therapy was found to have an unusually high requirement for I.F. This patient failed to absorb vitamin B₁₂ with 50 mg. of I.F. 186-1 and with 5 mg. of a more purified preparation (I.F. WES 727). However, 500 mg. of 186-1 and 50-100 mg. of WES 727 cause optimum absorption (Table V). This patient excreted 6.4 and 11.5% with 30 and 50 ml. respectively of fresh acid normal human

TABLE V.—Unusually High Intrinsic Factor Requirement
(Patient J. S.)*

Intrinsic Factor Source	Amount	Urinary Excretion (%)
None (B ₁₂ alone) ..	—	0.1, 0.2
186-I (crude) ..	50 mg.	2.2, 1.7, 0.9
	500 "	7.7
727 (purified) ..	5 "	0.2
	50 "	11.0
	100 "	19.0
Acid human gastric juice	30 ml.	6.4
	50 "	11.5

* We acknowledge the co-operation of Dr. Jerome Adelman, of Brooklyn Jewish Hospital, Brooklyn, N.Y., for permission to study this patient.

gastric juice. This, taken together with the fact that Patient 9 had a low excretion with I.F. prior to any oral therapy, emphasizes the importance of conducting control absorption studies on the same patients prior to oral therapy.

The 2 to 3 U.S.P. units used in the urinary excretion tests in the present report could not be classified as a large dose. In most of the reports describing refractoriness, doubling or tripling the dose was not adequate to restore absorption where absorption was blocked. The necessity for using an amount of intrinsic factor contained in 2 to 3 U.S.P. units in the urinary excretion test with 2 µg. of radio-labelled vitamin B₁₂ has been pointed out in previous communications (Williams *et al.*, 1956; Ellenbogen and Williams, 1958). Furthermore, the same amount of I.F. was used in the urinary excretion test at the beginning of the maintenance study as at the completion of the study with I.F. WES 592.

There appears to be no outstanding explanation why only one patient (No. 6) out of 24 patients showed a reduction in absorption of vitamin B₁₂. She was an extremely uncooperative subject, but a repeat urinary excretion test gave the same result. Possibly some type of latent malabsorption was developing.

Summary

Nine P.A. patients maintained on different B₁₂-I.F. preparations for 5 to 36 months, and then for 7 to 18 months on a single vitamin B₁₂-I.F. preparation equivalent to 2 U.S.P. units, failed to develop any resistance to the later preparation.

Only one out of 24 patients showed any significant indication of reduced absorption after oral maintenance for from 5 to 20 months on a preparation equivalent to 1 U.S.P. unit.

One P.A. patient who had never received oral therapy required approximately 20 times the usual amount of I.F. for optimum vitamin B₁₂ absorption.

It is concluded that some I.F. preparations can successfully maintain P.A. patients for long periods without showing impaired absorption of vitamin B₁₂.

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Medical Memoranda

Alveolar Soft Part Sarcoma

Alveolar soft part sarcoma, or metastasizing non-chromaffin paraganglioma, is a rare tumour with a distinct histological and clinical picture. Smetana and Scott (1951) and Christopherson *et al.* (1952) have each recorded a small series of cases; another case was described by Randall and Walter (1954). A further case is reported below.

CASE REPORT

In November, 1953, a woman aged 20 was referred to the chest clinic from the mass radiography unit. She complained of cough and lassitude, and there was a family history of tuberculosis. Chest x-ray examination showed a partly calcified focus beyond the left hilum. Thereafter she was observed regularly. In September, 1954, a round focus was noted in the left lower lobe; shadows appeared later at the left base (Fig. 1), and in July, 1955, she was admitted to Bow Arrow Hospital under the care of Dr. Michael Gibson. She was Mantoux-positive, and was treated with streptomycin and isoniazid; but doubt was expressed about the nature of the lesion. She returned home, and in November, 1956, attended the chest clinic when eight months pregnant; round foci were present in both lower lobes, and she was admitted to Grove Park Hospital. She had now developed a firm, painless swelling 2 by 3 in. (5 by 7.5 cm.) in the left erector spinae muscle. She was delivered of a healthy child on January 9, 1957.

FIG. 1.—Radiograph of chest (May, 1955).