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MAINTENANCE THERAPY OF PERNICIOUS ANÆMIA WITH ORAL ADMINISTRATION OF INTRINSIC FACTOR AND VITAMIN B_{12}*

LOUIS LOWENSTEIN, M.D.,
LAUDER BRUNTON, M.D.,
LORNE SHAPIRO, M.D.,
NANNIE DE LEEUW, M.D. and
MAURICE DUFRESNE, M.D., *Montreal*

SMALL AMOUNTS of vitamin B_{12} plus intrinsic factor from hog pyloric mucosa have been used in the treatment of pernicious anæmia for several years.[1, 1a] In 1956, we presented an initial report on the long-term oral therapy of 37 cases of pernicious anæmia with vitamin B_{12} combined with a concentrate of hog pyloric mucosa of proved potency as a source of intrinsic factor (Ayerst 5310 or "Cycoplex", M.C.D.).[2] Since then one of these patients has been dropped from the series. This therapy was maintained subsequently for a total period of three years in this group of patients, and it is the purpose of this paper to report the results of these studies.

MATERIAL AND METHODS

The therapeutic preparation consisted of capsules, each containing 5 μg. of vitamin B_{12} and 50 mg. of defatted, desiccated hog pyloric mucosa. These capsules were stored at 4° C. until issued to the patients every two to four weeks and the patients were instructed to refrigerate them.

The study was started in January 1954, with a total of 40 patients; four of these were dropped from the series for various reasons. One patient was dropped because of carcinoma of the stomach, another because of Paget's disease producing neurological signs similar to subacute combined degeneration of the cord; another died of streptococcal meningitis. Thirty-one of the patients included in this study had received previous treatment in the hæmatology out-patient clinic, consisting of 30 to 60 μg. of vitamin B_{12} parenterally every two to four weeks. The remaining five patients only received the oral preparation from their presentation in relapse through the maintenance period.

Patients were divided into four groups, of which Groups I, II and III were chosen at random. The patients of Group IV were selected because of the presence of complications which made larger therapeutic doses seem desirable. Patients in Group I received one capsule every three days, those in Group II received one capsule daily, those in Group III received two capsules daily, and those in Group IV received three capsules daily. Repeated questioning of the patients indicated that they took their capsules regularly.

Pre-treatment assessment consisted of clinical and hæmatologic examination of all patients, and marrow aspiration when indicated. Subsequent clinical and hæmatological examinations were performed at least once monthly. Sternal marrow aspirations were done on every patient after two years of oral therapy, or before this interval if indicated, and serial marrow aspirations were obtained in a number of patients.

Routine examination of the blood included hæmoglobin, packed cell volume, red cell count and examination of the blood film. Blood volume determinations were performed with RISA approximately two years after oral therapy was started, but only confirmed the presence of anæmia already revealed by routine hæmatologic procedures.

Serum iron and unsaturated iron binding capacity, and iron stains of the bone marrow were performed in certain patients who developed anæmia and who were suspected of having developed a concomitant iron deficiency. Iron therapy was given to the occasional patient in whom iron deficiency was demonstrated.

Serum vitamin B_{12} determinations were performed in all patients after two years of oral therapy, earlier in some cases, and also were performed serially in most of the patients.*

After completion of the three-year maintenance period on oral therapy and after the patients had been returned to parenteral vitamin B_{12} therapy, Schilling tests were performed in approximately one-half of the subjects, first with vitamin B_{12} alone using 0.5 μg. Co^{58} labelled vitamin B_{12} of high

*From the Hæmatology Services of the Departments of Medicine of the Royal Victoria Hospital and the Queen Mary Veterans Hospital, Montreal.
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specific activity and subsequently with vitamin B_{12} plus intrinsic factor using 100 mg. of desiccated, defatted hog pyloric mucosa as a source of intrinsic factor (supplied by Ayerst, McKenna & Harrison, Limited).†

RESULTS

TABLE I.

Group	No. of patients	Dosage (capsules)	Relapses		
			Clinical	Anæmia	Marrow
1	9	1 q.3.d.	0	1	7
2	10	1 daily	0	2	8
3	7	2 daily	0	0	2
4	10	3 daily	0	1	2
Total	36		0	4	19

1 capsule contains 5 μg. of vitamin B_{12} plus 50 mg. of hog's pyloric mucosa.

Table I summarizes the results. There were no objective clinical or neurological relapses during the three-year period. Four patients developed mild to moderate anæmia; three of these received the lower dosage schedules of Groups I or II. Bone marrow relapses occurred in 19 of the 36 patients; 14 of these were in Groups I and II and hence were on relatively low dosage.

Bone marrow changes consisted of intermediate megaloblastic erythropoiesis and macrogranulocytosis.

Tables IIA, B, C and D present the changes observed in the individual patients. The packed cell volumes are averages of the last three monthly determinations and were significantly reduced in the four patients who became anæmic. The blood of these patients became macrocytic as judged by the appearance of the red cells and calculation of the mean corpuscular volume. Occasional macrocytes were also observed in the blood smears of some of the other patients, but the red cells did not appear predominantly macrocytic and the mean corpuscular volume was less than 100 cu. microns.

In Fig. 1 the serum B_{12} results are arranged according to the four groups and are compared with the range and mean found in 150 normal subjects. Of the normal series, only two values were observed below 200 micromicrograms per ml. and the lowest was 130. In the great majority of observations the serum vitamin B_{12} concentrations of the patients of all four groups were

†A few of the Schilling tests were performed with Co^{60} labelled vitamin B_{12}.

TABLE IIA.—GROUP 1.

Patient	Sex	Hæmatocrit %	Macrocytosis	Marrow	Serum B_{12} μμg./ml. serial values
T.H.	M	43	0	MEG	135, 175, 137, *123*
J.L.	M	44	0	N	164, 118, 140, 217, 119, 154, 169
A.J.	M	47	0	MEG	*149*, 71, 67, 150, 79
G.M.	M	37	+	MEG	692, 1255, 1157, *1256*
S.M.	F	41	0	MEG	52, 53, 93, *99*
E.P.	F	44	0	MEG	123, 121, *213*
G.S.	M	45	0	MEG	120, 84, 69, *30*
M.S.	F	42	0	A	141, 127, 103, 134
S.W.	M	41	0	MEG	128, 194, 70, 96, *147*

Italic figures = Megaloblastic marrow at time of vitamin B_{12} determination.
N = Normal marrow.
MEG = Megaloblastic marrow.
A = Acellular marrow possibly due to radiation therapy for mammary cancer.

TABLE IIB.—GROUP 2.

Patient	Sex	Hæmatocrit %	Macrocytosis	Marrow	Serum B_{12} μμg./ml. serial values
J.A.	M	48	0	MEG	*32*, 99, 160, 405, *53*
I.C.	F	33	+	MEG	172, 142, *188*, 247
E.H.	F	40	0	N	229, 376, 222
G.K.	M	48	0	MEG	*106*, 127
C.J.	F	37	0	N	690, 345, 233, 307
W.J.	M	48	0	MEG	52, 103, *141* 1833
S.S.	F	40	0	MEG	181, 157, *182* 2182
E.S.	M	42	0	MEG	222, 152, 79, *114*
M.T.	F	42	0	MEG	75, *36*
*L.W.	F	31	+	MEG	*171, 81, 225, 216*

Italic figures = Megaloblastic marrow at time of vitamin B_{12} determination.
N = Normal marrow.
MEG = Megaloblastic marrow.
*Treated initially with oral B_{12} and intrinsic factor.

TABLE IIC.—GROUP 3.

Patient	Sex	Hæmatocrit %	Macrocytosis	Marrow	Serum B_{12} μμg./ml. serial values
S.A.	M	41	0	N	76, 209, 129, 133, 107
B.C.	M	48	0	MEG	240, 253, *<50*
H.F.	M	41	0	MEG	178, *118*, 87
J.H.	M	42	0	N	213, 127, 119, 93, 256
G.P.	M	43	0	N	115, 54, 157, 192, 147
E.R.	F	45	0	N	67
*A.W.	M	48	0	N	31, 33, 41

Italic figures = Megaloblastic marrow at time of vitamin B_{12} determination.
N = Normal marrow.
MEG = Megaloblastic marrow.
*Treated initially with oral B_{12} and intrinsic factor.

TABLE IID.—GROUP 4.

Patient	Sex	Hæmatocrit %	Macrocytosis	Marrow	Serum B_{12} μμg./ml. serial values
*M.C.	F	37	0	N	134, 48, 78, 106, 132
*P.C.	M	41	0	N	29, 21, 60, 67, 127
E.F.	M	48	0	MEG	34, 52, 92, 113, 72, *85*
S.D.	F	36	+	MEG	76, 106, 57, 65, *40*
J.P.	M	46	0	N	267, 356
E.H.	M	45	0	N	95, 213, 92, 276
W.S.	M	40	0	N	343, 539, 279, 107
W.T.	M	44	0	N	695, 709, 1299, 1687
D.R.	M	40	0	N	102, 100, 84, 360
*A.G.	F	40	0	N	71, 145, 139, 63, 124

Italic figures = Megaloblastic marrow at time of vitamin B_{12} determination.
N = Normal marrow.
MEG = Megaloblastic marrow.
*Treated initially with oral B_{12} and intrinsic factor.

less than 200 μμg./ml. There was no definite trend from Group I to Group IV which would relate higher serum vitamin B_{12} results to larger doses.

The serum vitamin B_{12} levels were consistently below 200 μμg./ml. in 20 of the 36 pernicious anæmia patients included in this study. In eight of the remaining 16 patients only one of the several serum vitamin B_{12} levels determined upon each of these patients was above 200

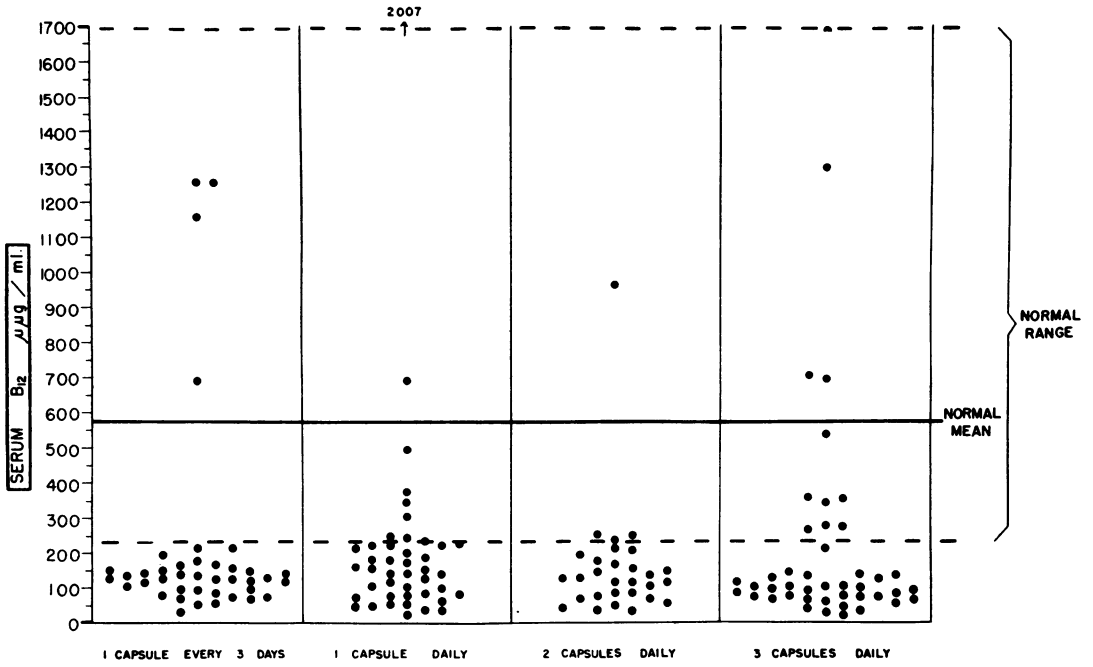

Fig. 1.—Serum vitamin B_{12} concentrations after 3 years' maintenance therapy. One capsule contains 5 μg. of vitamin B_{12} + 50 mg. of hog's pyloric mucosa.

μμg./ml. At the time of observation of megaloblastic relapse in the bone marrow the serum vitamin B_{12} levels were below 200 μμg./ml. in 17 patients. In one of the remaining two patients who relapsed (patient E.P., Group I) the serum B_{12} was 213 μμg./ml. at the time the bone marrow puncture showing megaloblastic relapse was performed. Previously, serum B_{12} levels of 123 and 121 μμg./ml. had been obtained in this patient.

Patient G.M. in Group I had very high values of vitamin B_{12} in the serum in the face of anæmia and frank megaloblastic bone marrow. The cause of this has not been determined.

Of the five patients treated wth the oral preparation initially and then maintained on this form of therapy, one patient relapsed. This patient (Patient L.W., Fig. 3) responded satisfactorily initially to three capsules daily, later relapsed on one capsule daily, and when again placed on three capsules daily, showed partial hæmatologic remission and slight improvement of serum vitamin B_{12} levels. Serum B_{12} levels in the other four patients of this group were consistently low, ranging from 21-145 μμg./ml. These four patients probably would have relapsed if oral therapy had been continued for a longer period. It would seem pertinent that, initially, one (A.G.) of these patients responded satisfactorily to one capsule every three days. Later she was placed on maintenance therapy of three capsules daily; although she had not relapsed hæmatologically at termination of the study, five serum vitamin B_{12} determinations varied from 63-145 μμg./ml. while she was on the larger dosage. The daily dose was increased to three capsules daily in two patients who relapsed on one capsule daily (patient S.D., Group IV) and one capsule every three days (patient E.F., Group IV). Both responded partially, but not well, to the increased dosage and their serum B_{12} level remained below 100 μμg./ml.

Thus, although initial hæmatologic response resulted from as little as one capsule every three days, an apparently refractory state often developed later, during which increased dosage of oral therapy produced either no remission or a partial and unsatisfactory remission and usually failed to elevate the serum vitamin B_{12} levels significantly.

Seventeen patients showed no evidence of relapse at the termination of the study. Ten of these had serum vitamin B_{12} levels well below 200 μμg./ml. The preliminary report of this study[2] included a survey of the findings to the end of June 1956. At that time nine patients showed marrow relapse. The majority of the patients had serum vitamin B_{12} levels below 200 μμg./ml. Between June 1, 1956, and January 1957, an additional ten patients developed evi-

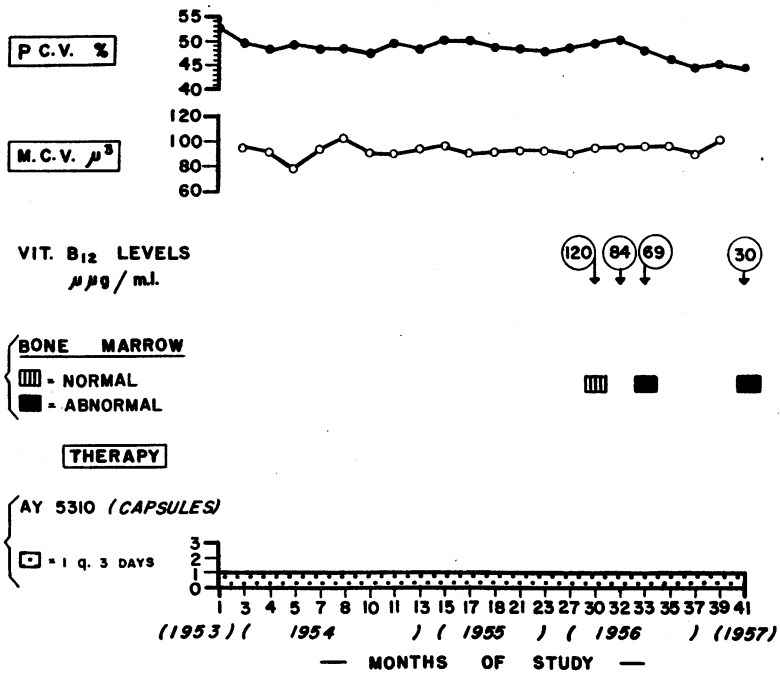

Fig. 2.—Case G.S., pernicious anæmia.

dence of relapse. The high relapse rate during this period was anticipated because of the low serum B_{12} level of the majority of the patients included in this study. Some patients had such low levels for many months before developing evidence of hæmatologic relapse in the bone marrow or blood, and at the time of discontinuation of the project a number of patients had maintained low levels for many months and still had not developed relapse.

The relationship of the hæmatologic findings to therapy is shown graphically for three patients. Patient G.S. (Fig. 2) developed a progressive fall in his serum vitamin B_{12} concentration to very low levels with eventual megaloblastic relapse of the bone marrow, but with no anæmia at the end of the experiment. The hæmatocrit did decrease from 53% to 44%, particularly during the last few months of the oral therapy, and MCV (mean corpuscular volume) increased slightly on the last observation. One cannot exclude the possibility that the decrease of packed cell volume indicated an early anæmia in this patient, although the actual levels were within the normal range. Patient L.W. (Fig. 3) was treated initially with the oral preparation and subsequently developed a satisfactory rise in reticulocytes and packed cell volume as well as a decrease of the MCV to the upper normal range. Her initial therapy consisted of three capsules daily and her maintenance therapy was one capsule daily. Twenty-six months later she was found to have a low serum vitamin B_{12} level and a megaloblastic marrow; at the same time she developed a significant drop in packed cell volume. She was then given three capsules daily and the packed cell volume and the serum vitamin B_{12} concentration improved. Although her bone marrow continued to show some megaloblastic transition, partial reversion to

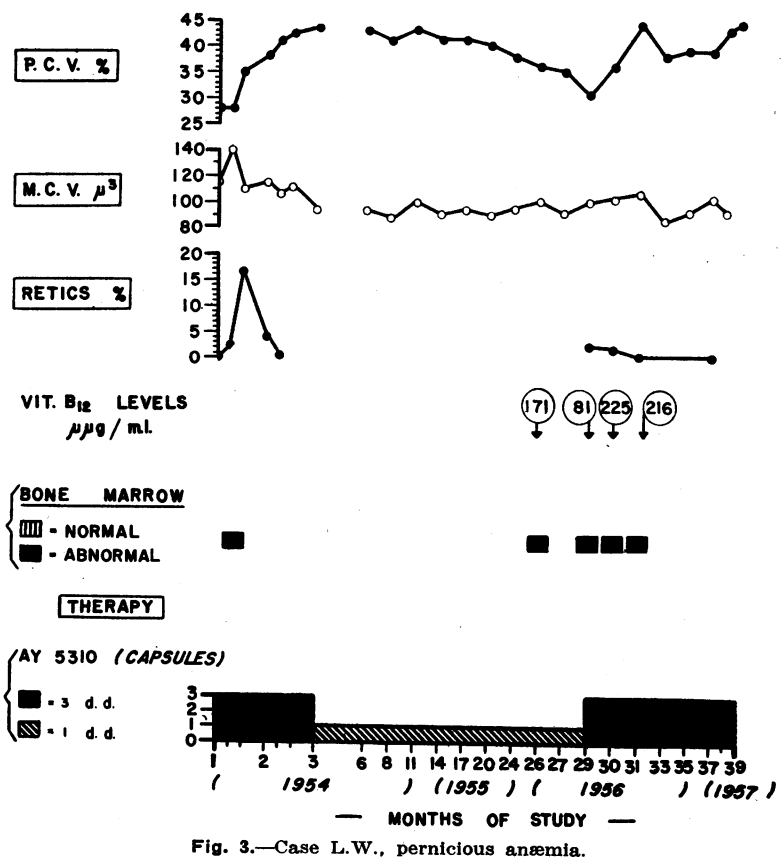

Fig. 3.—Case L.W., pernicious anæmia.

normoblastosis was present. Patient E.F. (Group IV, Fig. 4) developed thyrotoxicosis in the presence of a nodular goitre and the hæmatocrit value fell significantly. At the same time the serum vitamin B_{12} was 34 μmg./ml. and the marrow showed megaloblastic changes. The dosage of the oral preparation was increased from one to three capsules daily and subsequently the patient showed a slight reticulocytosis and increase of the packed cell volume, although the serum vitamin B_{12} level remained abnormally low and the megaloblastosis did not disappear from the bone marrow.

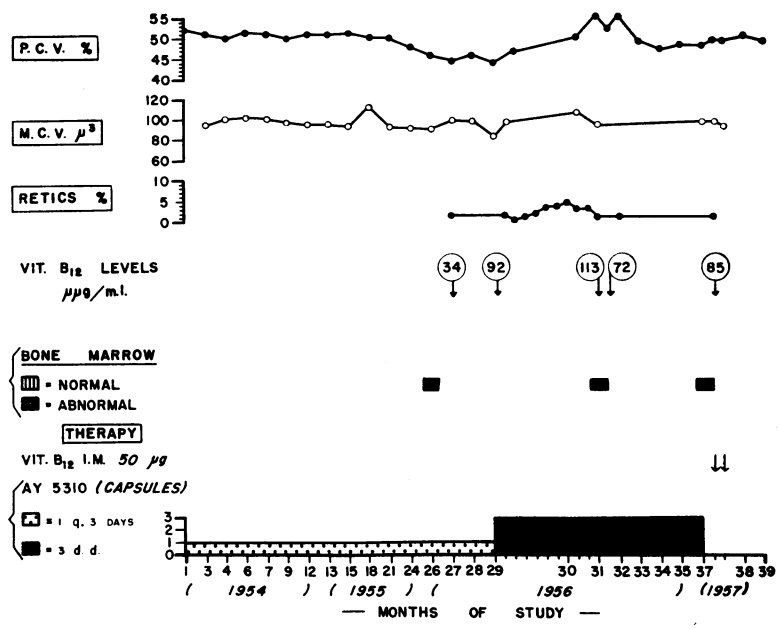

Fig. 4.—Case E.F., pernicious anæmia with thyrotoxicosis.

Since the termination of oral therapy, Schilling tests have been performed in 15 of these patients, first with radioactive vitamin B_{12} alone and then with radioactive vitamin B_{12} plus hog pyloric mucosa (Table III). With Co^{58} B_{12} alone the urinary excretion varied from 0 to 4.3%. When the test was repeated with the addition of hog pyloric mucosa, the urinary excretion varied from 0 to 24.9% of the ingested dose of Co^{58} B_{12}; in three instances the excretion was nil; in nine instances the excretion was less than 10%; in two instances the excretion was 10-15%; and in only four instances was the excretion over 15%. The tests were performed over a six-month period after termination of the oral therapy. When the test was performed in patients with pernicious anæmia who had not received the oral therapy and who received the same batch of Co^{58} B_{12} and of hog pyloric mucosa, over 15% of the ingested radioactivity was excreted in the urine. When the test was performed in normal controls with Co^{58} B_{12} without intrinsic factor, 18-30% of the ingested dose of radioactive B_{12} was excreted in the urine. These results indicate that the previous oral therapy had produced a functional state of variable refractoriness to the heterologous source of intrinsic factor in 11 of the 15 patients tested.

TABLE III.—RADIOACTIVE VITAMIN B_{12} ABSORPTION—24-HOUR EXCRETION IN URINE, PER CENT

Treated with oral preparation	*No intrinsic factor*	*With intrinsic factor, dried hog's pyloric mucosa*
D.R.	0	0
P.C.	2	0
W.S.	0	0
A.J.	0	0.7
S.A.	0	0.8
G.D.	0	5.2
H.F.	0	6.5
E.F.	0.35	7.8
M.T.	0	8.6
B.C.	0.43	10.8
A.G.	0.8	11.8
S.M.	1.9	15.9
J.P.	3.8	16.6
G.P.	0.33	19.6
M.S.	2.0	24.9
Parenteral treatment only		
J.W.	0	19.7
E.C.	4.1	16
M.S.	4.3	19.9
Normal		
M.C.	18.0	—
M.J.	25.8	—
C.G.	26.9	—
C.B.	30.0	—

DISCUSSION

Megaloblastic changes in the bone marrow proved the most sensitive indicator of relapse in this series of patients. This finding is in agreement with previous observations in patients with megaloblastic anæmia.[3-5] None showed evidence of clinical relapse. Definite hæmatologic relapse of the blood occurred in only four patients over the three-year period. Nineteen patients developed bone marrow relapse. An additional 10 patients had significantly low serum vitamin B_{12} levels with normal blood

and bone marrow and with no objective evidence of clinical relapse. It is probable that some of these 10 patients would have developed megaloblastic changes in the bone marrow had the oral therapy been continued for longer than three years.

A number of factors must be taken into consideration in evaluating the efficacy of this form of therapy and the development of relapse in this group of patients.

The hog pyloric mucosa preparation used as a source of intrinsic factor in these experiments has been shown by others, when taken orally in conjunction with small amounts of vitamin B_{12}, to produce satisfactory remission of pernicious anæmia in relapse.[1, 6] Five of the patients of this series were initially treated with the oral preparation and developed satisfactory hæmatologic remission. Consequently, it may be assumed that the preparation of intrinsic factor was an active one.

As maintenance therapy was continued, relapse developed in an increasing number of patients. A number of observers have called attention to the development of relapse after initial response in patients receiving oral vitamin B_{12} and hog pyloric mucosa as a source of intrinsic factor.[1a, 2, 7-9] This has occurred both with crude and with relatively pure preparations of the active principle. Recently, Schwartz[8] and Killander[9] have shown that this reduced effect is probably due to development of refractoriness to intrinsic factor of heterologous origin. When hog pyloric mucosa was used as a source of intrinsic factor in the Schilling test by these observers, untreated pernicious anæmia patients showed normal urinary excretion of ingested radioactive B_{12}. When, however, the test was performed in pernicious anæmia patients who had previously received oral treatment with a preparation of B_{12} and hog pyloric mucosa, the urinary excretion of radioactive vitamin B_{12} was markedly diminished due to decreased absorption of the B_{12}. Substitution of normal human gastric juice for the hog pyloric mucosa resulted in normal absorption of the radioactive vitamin B_{12} in the patients who had received oral treatment with B_{12} and hog pyloric mucosa.

The Schilling test was performed in over half of the patients of our series during the six months which followed termination of this form of therapy.* The majority of these patients showed reduced excretion of the radioactive B_{12} when a preparation of hog pyloric mucosa was used as a source of intrinsic factor. On the other hand, when the same source of intrinsic factor was used in the Schilling test in conjunction with Co^{58} B_{12} in patients who had not been treated with the oral form of therapy, normal absorption of the radioactive vitamin B_{12} occurred. Our results agree with those of Schwartz and of Killander. Consequently, one probable reason for the high relapse rate of this group of patients was the development of a state of relative refractoriness to the intrinsic factor effect of the hog pyloric mucosa. It has been stated that the level of vitamin B_{12} in the serum is not necessarily an accurate indicator of the state of saturation of the tissues with this substance.[10] Glass[11] noted that often the low pre-treatment levels of serum vitamin B_{12} were not altered in pernicious anæmia patients, although they responded hæmatologically to oral therapy. The hæmatologic response of the blood and of the bone marrow is probably a more sensitive indicator of response than are changes in the serum vitamin B_{12} level.[12] Hence, it would seem possible for enough of vitamin B_{12} to be absorbed during oral administration to prevent or retard hæmatologic relapse, although the serum vitamin B_{12} dropped to very low levels. Consequently, the low serum vitamin B_{12} levels alone may not have been sufficient evidence to indicate the existence of relapse in these patients. Low levels have been observed for many months without the development of megaloblastic relapse in pernicious anæmia.[7]

Lowther[13] reported results of six months of maintenance therapy in 20 patients who received oral vitamin B_{12} and hog pyloric mucosa intrinsic factor (Bifacton). None of their patients showed clinical or hæmatologic relapse during this six-month period. It is noteworthy that bone marrow aspiration was not used as a criterion. These authors quote the work of Schwartz and Leger, who followed the course of pernicious anæmia patients in whom treatment was stopped, and noted that 33% relapsed during the first six months without treatment and another 36% during the second six months. The use of these data of Schwartz

*See Appendix.

and Leger to indicate that the oral form of therapy was effective in a group of patients studied by Lowther would seem open to criticism. Small amounts of B_{12} may have been absorbed by Lowther's patients. This supply although inadequate to satisfy the needs of the body may nevertheless have been sufficient to delay relapses beyond the six-month period. In addition, the state of the bone marrow was not ascertained by this group of observers. Furthermore, the liver alone normally stores between 1000 and 2000 μg. of vitamin B_{12},[5] and normally enough vitamin B_{12} is stored in the tissue depots of the human to meet the body needs for two to four years.[11, 14, 15] If the original parenteral therapy had succeeded in building roughly normal stores of vitamin B_{12} in their tissues, the patients studied by Lowther would have required a follow-up period of more than six months before effectiveness of oral maintenance therapy could have been determined. The majority of the 19 relapses which occurred in our patients only became evident after two to three years of oral therapy.

A number of observers have confirmed Goldhamer's observation that the deficiency of intrinsic factor in pernicious anæmia is a relative deficiency and its extent is subject to individual variations.[16, 17] More recently, Baker[18] has shown that with administration of adequate amounts of intrinsic factor some patients with pernicious anæmia absorb only very minute amounts of vitamin B_{12}. Thus there appears to be a considerable gradient in pernicious anæmia patients, not only in their degree of intrinsic factor deficiency, but also in the enhancing effect of this intrinsic factor upon the absorption of vitamin B_{12}. Consequently when one considers the administration of intrinsic factor to facilitate the absorption of vitamin B_{12} one must consider the degree of intrinsic factor deficiency, the development of refractoriness to heterologous intrinsic factor and possibly the state of an intestinal acceptor mechanism which is concerned with the absorption of vitamin B_{12} in the presence of adequate amounts of intrinsic factor.[14, 19, 20]

It will be noted that one patient developed hæmatologic and bone marrow relapse, although the serum vitamin B_{12} levels were abnormally high. The occurrence of inexplicably and unpredictably high serum vitamin B_{12} concentrations has been noted by a number of authors.[21] This patient showed no clinical or laboratory evidence of leukæmia or of diffuse liver disease, conditions in which high serum vitamin B_{12} levels have been noted.[23-25] The presence in the patient's serum of some factor other than vitamin B_{12} which is effective in promoting the growth of *L. leischmanii* must be considered as a possibility. Another possibility could be that most if not all of the serum vitamin B_{12} in this patient existed in the combined form and was bound to some abnormal protein in such a way that the B_{12} was functionally unavailable to the body.

Although a few patients relapsed on oral therapy and then partially responded to larger doses, their response was in no case completely satisfactory. Consequently it is likely that a state of complete or partial refractoriness to the heterologous source of intrinsic factor developed in them.

The findings of a low serum vitamin B_{12} level for many months in some patients before the development of megaloblastic relapse of the bone marrow agrees with the observations of others.

SUMMARY AND CONCLUSIONS

1. Thirty-six patients were maintained for a three-year period on oral administration of daily doses of vitamin B_{12} and hog pyloric mucosa as a source of intrinsic factor.

2. At the termination of the three-year period, 19 patients had developed megaloblastic relapse of the bone marrow and four patients had developed definite evidence of relapse of the blood, although no objective clinical relapses were observed. An additional 10 patients exhibited abnormally low serum vitamin B_{12} levels.

3. Schilling tests, using Co^{58} B_{12} alone and Co^{58} B_{12} with hog pyloric mucosa as a source of intrinsic factor, were performed in over half the patients. In the majority of those tested some degree of refractoriness to the heterologous intrinsic factor had developed after the three-year period of oral administration.

4. Oral administration of small amounts of vitamin B_{12} with desiccated hog pyloric mucosa as a source of intrinsic factor is not a satisfactory method of maintenance therapy in Addisonian pernicious anæmia.

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RÉSUMÉ

On présente ici une série de 36 malades atteints d'anémie pernicieuse et maintenus pendant une période de trois ans à une thérapeutique orale comprenant de la vitamine B_{12} et du facteur intrinsèque extrait de muqueuse pylorique de porc. A la fin de cette période d'observation, une rechute s'était produite chez 19 malades qui montraient une mœlle osseuse mégaloblastique et 4 autres dont les altérations hématologiques s'étendaient au sang périphérique. Aucun signe clinique de rechute cependant ne put être décelé. Dix autres malades montrèrent un taux sérique de vitamine B_{12} très bas. L'épreuve de Schilling basée sur la comparaison de l'excrétion de vitamine B_{12} marquée au Cobalt 58 avec et sans l'administration de facteur intrinsèque, fut pratiquée dans plus de la moitié du groupe. L'examen révéla chez la plupart de ceux qui le subirent un état réfractaire à l'égard du facteur intrinsèque hétérologue qu'ils avaient reçu *per os* pendant une période de trois ans. Ce recul permet d'affirmer que de petites quantités de vitamine B_{12} administrées oralement avec de la muqueuse desséchée de pylore de porc comme source de facteur intrinsèque n'est pas un traitement d'entretien satisfaisant dans la maladie de Biermer.

APPENDIX:

Since this paper was submitted for publication Schilling tests as described in the text of the paper have been performed upon additional patients of this series, bringing the total number to 32. After ingestion of vitamin B_{12} alone, 31 of the 32 patients excreted 5% or less of the ingested dose (the other patient excreted 9.2% and is being reassessed). After the administration of Co^{58} B^{12} and hog pyloric mucosa as a source of intrinsic factor, excretion was as follows:

Percentage	*No. of patients*
0-1	11
1.1-10	10
10.1-14.9	4
15+	7
	32

From the available data it is impossible to determine whether the relapses which occurred in Groups I and II were due to low dosage and/or to the development of poor absorption of vitamin B_{12}. In Group IV, in which the highest dosage was administered, the development of relapse could be correlated with the results of the Schilling tests. Repeat tests in a number of patients of this series have shown persistent impairment of B_{12} absorption, although oral therapy was terminated nine months ago. These tests have also been performed upon an additional number of normal controls and pernicious anæmia patients receiving parenteral treatment alone. The results in these two latter groups did not differ from those recorded in similar patients in Table III.

CONTROL OF ETHYL BISCOUMACETATE (TROMEXAN) THERAPY BY STANDARDIZED CLOTTING TIME OF WHOLE BLOOD*

GEORGE A. MAYER, M.D. and W. FORD CONNELL, M.D., *Kingston, Ont.*

THE CLOTTING TIME of whole blood has long seemed to many a rational measure of the effects of orally given anticoagulants, but early studies failed to establish any significant effect of these drugs on available procedures. It is true that Davidson and MacDonald[1] and Kadish[2] did demonstrate during dicoumarol therapy a prolongation of the clotting time in lusteroid tubes. Further, Moloney[3] and Margulies and Barker[4] showed a relationship between the clotting time in silicone-coated tubes and dicoumarol therapy. By these techniques, the clotting times were inconveniently prolonged and the end-point was indefinite. None of the authors named suggested that coumarin therapy could be so controlled.

Four years ago, one of us (G.A.M.) developed a highly reproducible procedure for the determination of the clotting time of whole

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