SINGLE MASSIVE DOSE OF VITAMIN B_{12} IN UNTREATED PERNICIOUS ANAEMIA

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The efficacy of parenterally administered vitamin B_{12} in microgramme doses has been unequivocally shown by Ungley in pernicious anaemia (1949a) and subacute combined degeneration (1949b), and the response of the former to varying doses quantitatively assessed (1949a). Adequate initial and maintenance dosage has been determined for both of these conditions (Ungley, 1950, 1951). The object of this study was to determine whether, in untreated pernicious anaemia, a single dose of vitamin B_{12} greatly in excess of that found to give the optimal initial response would result in a prolonged remission and thereby furnish presumptive evidence of the effective storage of vitamin B_{12} in the body following injection.

In carrying out this object a point of great interest was the duration of the normoblastic response in the marrow following a 1-mg. dose, and the comparison of this with the duration of normal findings in the peripheral blood and the clinical state of the patient. Certain difficulties exist from relatively unknown factors such as spontaneous remissions, but in spite of this the findings in blood and marrow are of much interest, and may throw some light on the question of whether in the treatment of pernicious anaemia larger injections at longer intervals than hitherto are feasible.

Methods

The series comprised 15 patients who were each given a single intramuscular injection of 1 mg. of crystalline vitamin B_{12}. The clinical and haematological findings in each patient fulfilled the accepted criteria for the diagnosis of pernicious anaemia, the most important being macrocytic anaemia, megaloblastic marrow, histamine-fast achlorhydria, normal barium-meal examination, and at least three consecutive negative tests for blood in the stools. When the anaemia was not severe the patients were observed without treatment for periods varying from two to six weeks to exclude a spontaneous remission.

Following the injection, favourable reticulocyte and red-cell responses were obtained in every case, and after discharge from hospital the patients were kept under regular review for clinical and blood examination, as nearly as possible at intervals of one month from the date of injection. On the whole, attendance at the appropriate intervals was good, although there were occasional gaps in the records. The patients themselves and their family doctors were requested to avoid any anti-anaemia treatment other than that given at the follow-up clinic, and we are satisfied that no other treatment was given. No special diet was prescribed after discharge from hospital, and the only additional treatment given was oral iron in full doses to those patients who showed evidence of iron deficiency at any stage. The criteria for relapse in the peripheral blood were a fall in the red-cell count, generally below 4.5 millions, and/or a large or persistent rise in the mean corpuscular volume. Examinations were repeated as necessary when there was reasonable doubt about the significance of the findings. Symptoms such as sore tongue and paraesthesiae would have been taken as indications of relapse, but in fact did not occur when the blood was normal except in one patient just before relapse became evident in the blood.

A further marrow examination was done at least once in each patient, and the time of these marrow studies ranged, in different cases, from 69 to 358 days after the single injection of vitamin B_{12}. Differential counts (400 cells) of the red-cell precursors in these marrows (see Table I) were done, using the classification and nomenclature given by Whitby and Britton (1950), and the appearance of the cells placed in each category follows in general their description, except that the cells classified as late normoblasts had nuclei that were pyknotic or very nearly so. The group of intermediate normoblasts, therefore, is the most heterogeneous one as regards varying degrees of maturation in the cells included in it, and corresponds to the early and late polychromatic normoblasts described by Dacie and White (1949). Like these authors, we have found that this is generally the predominating red-cell precursor in normal marrows.

In the follow-up marrows of this series, with their frequently minor variations from normality, considerable difficulty was sometimes experienced in differentiating promegaloblasts from pronormoblasts as described by Dacie and White (1949). Because the early megaloblast is easily recognized, and as distinction at an earlier phase was not important for our purpose, it was not given a place in the differential counts (see Table I). One type of cell, however, that we, like others such as Mollin and Dacie (1950), have found of great value in detecting slight or partial tendencies to megaloblastic haemopoiesis is the transitional

TABLE I.—*Differential Counts, 400 Cells, of the Red-cell Precursors in 18 Marrow Examinations Done on 14 Patients at Different Time Intervals Following a Single Injection of 1 mg. Vitamin B_{12}. The Time Elapsing Until Detection of Relapse in the Peripheral Blood is Also Given. Where the Space is Blank the Patient is Still in Remission.*

Patient:	1*	2	2	3	3*	4	5	5	5*	6	7	8	9	10	12	13	14	15
Initial R.B.C. (millions per c.mm.)	3·35	1·05		1·9	3·3	0·79	2·1	2·1	3·13	1·64	0·8	1·28	1·9	1·51	1·07	0·8	1·28	2·3
No. of days to marrow examination	93	116	358	107	192	76	69	137	98	110	115	95	150	81	86	95	92	82
No. of days to relapse in peripheral blood	348			150	192	128	132	132	142	182	170	181		81				
Proerythroblasts (%)	2	2	2·5	0·5	4·25	1	2	5	4	4·25	2·5	1·5	0·5	2	2·25	3·25	1·5	1·25
Early (basophil) normoblasts (%)	6	7	5	9·5	2	5	3·5	3·25	6·5	6·75	7	12·5	4·5	2·5	4	7·25	1·75	4
Intermediate (polychromatic) normoblasts (%)	49·5	44·5	45·5	42	25·75	49·5	46	12·5	32·5	31	29·25	46·75	50·5	4	45	41·5	47·75	51·5
Late (pyknotic) normoblasts (%)	42·5	46·5	46·75	45	25	41·5	48·5	15	57	22·5	38	38·5	44	8·5	44·25	38	49	42·75
Early (basophil) megaloblasts (%)	0	0	0	0	1	0	0	9·5	0	2·5	1	0	0	11·25	0	0·5	0	0
Intermediate (polychromatic) megaloblasts (%)	0	0	0	0	4	0·25	0	29·5	0	7	6	0	0	51·75	0·25	0	0	0
Late megaloblasts (%)	0	0	0	0	6	0·5	0	6·5	0	4	4·25	0·25	0	10	0·25	2	0	0
Transitional erythroblasts (%)	0	0	0·25	3	28	2·25	0	18·75	0	22	12	0·5	0·5	10	4	7·5	0	0·5

* After second injection of 1 mg. vitamin B_{12}.

erythroblast, recently described by Israëls (1951), and also by Dacie and White (1949) in their review, under the name of "intermediate megaloblast." As this term would lead to obvious confusion with the ripening frank megaloblast in the classification adopted in this study we have used the term "transitional erythroblast." These cells have appearances, especially in the nuclear chromatin, that are intermediate or transitional between those of a frank normoblast and a frank megaloblast.

The red-cell precursors in some of these marrows, both before and after treatment, were also counted, following the simpler classification suggested by Davidson *et al.* (1942). With this method it was found that gross megaloblastic change, and the alterations in the marrow 24–48 hours after injection described by these authors, were easily detected, but that lesser though definite megaloblastic changes were not revealed in such a classification. Examples of five treated and one initial marrow counted in this way are given for comparison (see Table II).

TABLE II.—*Differential Counts of Red-cell Precursors in Marrow Following Classification of Davidson et al. (1942)*

Patient	Days from Injection	Type I %	Type II %	Type III %	Type IV %
2	116	0·5	5·5	44·0	50·0
3	107	1·5	12·0	38·5	48·0
4	76	0·5	8·0	47·5	44·0
8	95	1·5	9·0	38·0	51·5
9	150	3·0	16·0	38·5	42·5
15	Initial untreated	33·5	32·5	24·0	10·0

Results

(1) Peripheral Blood

Erf and Wimer (1949) concluded from studies in four untreated patients that, in pernicious anaemia in relapse, remission could be maintained for 50–100 days with doses of 50–100 μg. vitamin B_{12}. This amount, however, was not always given in a single dose, and their criterion of remission was apparently symptomatic only, for in none of their patients was a red-cell count of over 4 millions recorded. In the patient followed for the longest period (114 days) the peak red-cell response was only 3.9 millions on the eighty-sixth day after a 50-μg. injection, and the red cells numbered only 3.2 millions and the haemoglobin was 68% on the 114th day. This is not surprising, as Ungley (1949a) found that the amount of vitamin B_{12} required (in divided and regular dosage) to raise the red cells to over 4.5 millions (that is, to complete remission) in 32 patients varied from 15 to 140 μg., and the time 15 to 118 days. Erf and Wimer also found that one patient required repeated doses because of severe neurological symptoms, persisting in spite of haematological improvement. They studied the effect of single doses of 32 μg. given to patients in remission for periods up to 140 days; but again their criteria for remission are vague, and the highest red-cell count recorded in these patients was 4.17 millions, the majority of counts being considerably lower.

Of the 15 patients of our series, 11 had complete remissions of varying duration following the injection of 1 mg. vitamin B_{12}, and one (No. 13) is still in the stage of improvement at the time of writing. One patient (No. 11) with subacute combined degeneration had to be given further injections at the end of six weeks because of neurological relapse; another (No. 4) did not achieve a higher red-cell count than 4.25 millions after the first injection, although remission was complete in other respects and was entirely satisfactory after the second injection of 1 mg., given on relapse at 128 days; while a third (No. 10), a man of 78 with a recent history of infective hepatitis and evidence of hepatic dysfunction, after an initial satisfactory response relapsed in 81 days. To date, eight patients have been followed till they showed signs of relapse after a first injection. In these, the time taken for relapse to show in the peripheral blood varied from 81 to 253 days. The short duration of response in patient No. 10 (81 days) may be due to defective storage of vitamin B_{12} in a damaged liver. Of the remaining patients, one (No. 2) is normal in blood and marrow nearly 12 months (358 days) after his single injection; another (No. 9) has normal blood findings after 251 days; while the four most recent patients have normal or improving blood at the end of 12 weeks.

Seven of the relapsed patients (Nos. 1, 3, 4, 5, 6, 7, 8) have been given a second injection of 1 mg. vitamin B_{12} at a time when the blood deficiency was slight. Patient No. 1 showed signs of relapse only after 348 days following this second dose, patient 3 after 192 days, patient 4 after 149 days, and patient 5 a probable relapse after 142 days. The others are still in remission at time of writing, 6–12 weeks after the second dose.

(2) Marrow

Mollin and Dacie (1950) found that to maintain a normoblastic marrow for 15 days after a single injection, doses as high as 30–40 μg. of crystalline vitamin B_{12} are required. With smaller doses, "average satisfactory" red-cell responses occur, but the marrow is megaloblastic before the end of 15 days. They emphasize the significance of "intermediate" (transitional) cells and the great sensitivity of these marrow changes, which vary in degree according to the severity of vitamin-B_{12} deficiency, of which they are the most delicate index. In this series (see Table I) three marrows, in different patients, were found to be completely normoblastic at intervals of 69, 92, and 358 days from the first injection, while three others, at intervals of 82, 95, and 150 days, showed changes from normoblastic haemopoiesis so slight as to be doubtful and negligible. Two further marrows were entirely normoblastic 93 and 98 days after the second injection of 1 mg. vitamin B_{12}, given on the first sign of relapse. Of the remainder examined after the initial dose, two showed very obvious megaloblastic change after 81 and 137 days, the first of these being the patient (No. 10) with hepatic insufficiency; while six showed minor but significant changes—from 3% to 35% of abnormal red-cell precursors—at 76, 86, 95, 107, 110, and 115 days after injection.

In all of these last six patients the clinical state and peripheral blood were normal (or, in the case of patient 13, still improving) at the time of the marrow examination, and a point of interest is the time that passed after the discovery of small though definite marrow abnormalities before signs of relapse became evident in the peripheral blood. In four of these patients the lag period between marrow and blood relapse was 43, 52, 72, and 55 days; the two others, more recent patients, have yet to show relapse in the blood. The two patients with a time-lag between marrow and blood abnormalities of 72 and 55 days defaulted for a while in attendance, else the interval might have been shorter. In spite of this it would appear that indications of megaloblastic haemopoiesis may be found in the marrow 40 to 50 days before any change is discovered in the blood. This is in accordance with the opinion of Mollin and Dacie (1950), that marrow changes are the most sensitive index of vitamin-B_{12} lack. In seven patients with normoblastic marrows (eight examinations) on follow-up, the time elapsing in two before blood relapse was discovered was 255 and 86 days, in a third (patient 5) 63 and 44 days, while four (2, 9, 14, 15) are still in remission (see Table I).

(3) Clinical and Neurological State

The general symptoms associated with anaemia improved in every patient with the improvement in the blood. Except in patients 10 and 11, there was no serious complicating disease. Only one patient (No. 7) complained of slight lassitude at the time when relapse was discovered in his blood. The others continued to feel normal. With one important exception (No. 11), none of these patients showed any evidence of subacute combined degeneration, either initially or while under observation, after one injection of 1 mg. vitamin B_{12}. Three (Nos. 3, 4, 8) had slight initial paraesthesiae confined to the tips of fingers or toes, and three

(Nos. 4, 7, 8) had initial sore tongue. These symptoms had complete relief. One patient (No. 15) had a severe stomatitis, which had resisted many forms of treatment but improved rapidly after the injection. Only one patient (No. 1) developed slight paraesthesiae while under observation, at a time when the blood showed evidence of relapse eight months after the injection. The symptoms disappeared after the second injection and did not return within 49 weeks.

Summary

Fifteen patients with untreated pernicious anaemia were given a single parenteral dose of 1 mg. of vitamin B_{12} and followed until relapse. The clinical state, blood, and marrow remained normal in one case for as long as 358 days after such a dose, but the earliest relapse occurred in 128 days, excluding one case with hepatic insufficiency where it occurred at 81 days.

The marrow showed minor signs of reversion to megaloblastic haemopoiesis 40–50 days before there was any sign of relapse clinically or in the blood.

No untoward neurological or other clinical symptoms were encountered in any patient with initially uncomplicated pernicious anaemia, but the one patient in the series with subacute combined degeneration had to have intensive treatment resumed at the sixth week because of neurological deterioration.

While of theoretical and practical interest, these findings do not justify, without further trials, the routine treatment of pernicious anaemia by 1 mg. doses of vitamin B_{12} at long intervals.

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The renaming of Bristol's new health centre after Dr. William Budd will keep alive the memory of a great general practitioner and a pioneer epidemiologist. Budd was one of the first to distinguish between typhoid fever and typhus, and his views on public sanitation, particularly on the importance of a pure water supply, were well ahead of his times. In 1841 Bristol had been declared by a Government commission "the third most unhealthy town in England," surpassed only by Liverpool and Manchester. There was indeed scope in Bristol for Budd's genius. During the 1849 cholera epidemic he recommended rational methods of prevention—the disinfection or destruction of dejecta and fomites, and the provision of pure water. A curious and little-known incident of Budd's later life is recorded in Dr. E. W. Goodall's book, *William Budd* (Arrowsmith, Bristol). Budd was well liked and respected by his colleagues, but once he incurred their severe censure. An article on scarlet fever, somewhat critical of current medical practice and over his own name, appeared in the *Bristol Times and Mirror* the day before it was published in the *British Medical Journal*. A meeting of the Faculty at Bristol, held in Budd's absence, declared this to be "inconsistent with professional propriety."

RENAL CIRCULATION AND CARDIAC OUTPUT IN "LOW-OUTPUT" HEART FAILURE AND IN MYXOEDEMA

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It has been shown in a previous paper (Davies and Kilpatrick, 1951) that the cardiac output, the renal blood flow, and the glomerular filtration rate are reduced in congestive heart failure due to chronic rheumatic heart disease ("low-output" heart failure): the renal blood flow is reduced to a greater extent proportionately than the cardiac output and glomerular filtration rate. Merrill (1946) and Mokotoff, Ross, and Leiter (1948) postulated that retention of salt in low-output heart failure results from a low glomerular filtration rate in association with normal tubular reabsorption, but evidence has been adduced (Davies and Kilpatrick, 1951; Davies, 1951) which suggests that, although low glomerular filtration rate may be a contributory factor in salt retention, increased tubular reabsorption is more important.

The finding by Corcoran and Page (1947) of low renal clearances in two patients suffering from myxoedema suggested the present study, which is a comparison of the renal circulation, the cardiac output, and the ability to excrete salt in patients suffering from rheumatic heart disease and patients suffering from myxoedema. The former required regular administration of mercurial diuretics or severe restriction of salt intake in order to remain free of oedema. In addition, one patient with mitral stenosis *and* spontaneous myxoedema has been studied.

Normal standards for renal clearances were established in 14 healthy persons, and normal values for cardiac output were obtained from the work of McMichael and Sharpey-Schafer (1944).

Methods

The methods employed for the measurement of glomerular filtration rate (inulin clearance), renal plasma flow (*para*-aminohippuric-acid clearance), and renal blood flow $\left(\frac{\text{Renal plasma flow} \times 100}{100 - \text{Haematocrit}}\right)$ have been described previously (Dick and Davies, 1949; Davies and Kilpatrick, 1951). The validity of *para*-aminohippuric-acid clearance as a measure of renal plasma flow in patients suffering from myxoedema has not been established by determination of the concentration of *para*-aminohippuric-acid in renal venous blood. Cardiac output was measured by the methods of McMichael and Sharpey-Schafer (1944). The ability to excrete salt was measured by observing the effect of a diet containing 10 g. of salt and 1,500 ml. of fluid daily on the patients' weight.

Results

The findings in normal subjects, in patients with rheumatic heart disease, in patients with myxoedema, and in the single patient with both diseases are recorded in Tables I, II, and III.