been put out of action—for example, by drying as at B, or by heating to 45° C. as at C—gave an abundant growth of colonies all through the specimen. Finally, in order to find out whether the pus could deal with a greater number of microbes than it originally contained, other drops were placed on a heavily infected agar surface (see lower half of Fig. 5). Under the drop of fresh untreated pus (at D) there was complete suppression of growth; while under the dried pus (at E) there was no suppression. We were unable by this method to arrive at the limit of the antimicrobic power of the leucocytes. Wright gave the name "bio-pyo-culture" to this type of experiment.

By these and many other ingenious experiments Wright contributed more, in my judgment, to our understanding of the factors at work in an infected wound than anybody else in the last 100 years. Again he had "made bacteriology" and also immunology.

As his work developed he was able to formulate the lines along which "treatment by physiological agencies" should proceed. In the early stages, he told the surgeons, the emphasis should be on the removal of foreign bodies and

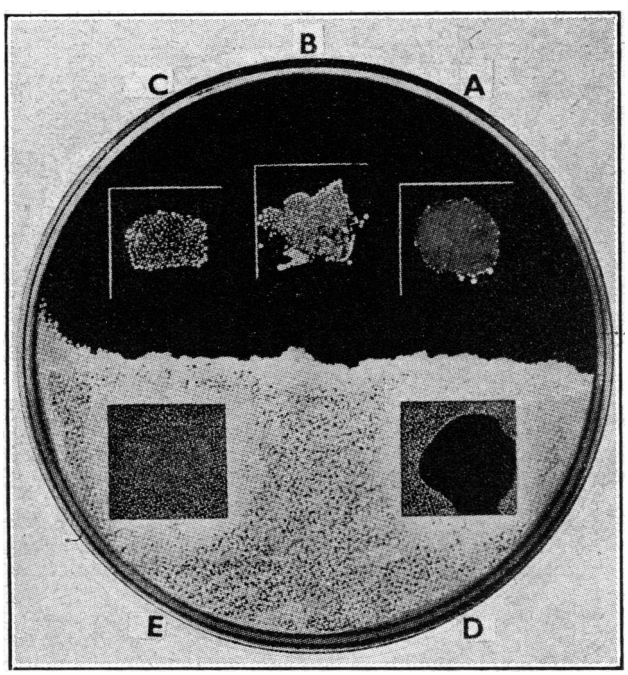

FIG. 5.—Experiment demonstrating the antimicrobic power of leucocytes.

necrotic tissues (since these would create "dead spaces") and on the promotion of an abundant flow of fresh lymph, rich in anti-proteolytic factor, to restrain the proliferation of microbes. In the later stages the emphasis should be on securing the best conditions for the functioning of leucocytes and on healing. "The leucocyte is the best antiseptic," he would say, and closure of the wound—by suture or skin-grafting—will usually give it the best conditions. In its essence, the present-day treatment of wounds finds its scientific basis in Wright's laboratory work during the first world war, although the textbooks of surgery tell the student nothing of that work.

I have spoken of only three points at which Wright broke new ground in medical science. There were of course many more. He was not greatly distressed that so much of his work failed to win general acceptance during his lifetime. He knew too well that the unconventional view is seldom popular, and he was confident that, in so far as he had found truth, his work would endure. "After all," he would say, "the Almighty is in no hurry; why should I be?"

Figs. 1, 2, and 3 are taken from Professor Fleming's paper in the *British Journal of Surgery*, 1919, **25**, 99; Fig. 5 from the paper by Almroth Wright in the *Lancet*, 1919, **1**, 489.

SERUM VITAMIN B_{12} CONCENTRATIONS OF PATIENTS WITH MEGALOBLASTIC ANAEMIA AFTER TREATMENT WITH VITAMIN B_{12}, FOLIC ACID, OR FOLINIC ACID

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The vitamin B_{12} concentrations in the serum and urine of normal subjects and of patients with untreated megaloblastic anaemia have already been reported (Mollin and Ross, 1952). Measurements of the vitamin were made by microbiological assay using *Euglena gracilis* var. *bacillaris* as test organism. The mean vitamin B_{12} concentration in the serum of normal subjects was found to be about 320 $\mu\mu$g. per ml.; the lower limit of the range in normal subjects was about 100 $\mu\mu$g. per ml. The concentrations in the serum of patients with untreated pernicious anaemia were below the normal range; the mean concentration was less than 40 $\mu\mu$g. per ml.

The changes in vitamin B_{12} concentrations in serum and urine during the first 72 hours after intramuscular injections of vitamin B_{12} have also been described. After the injections the concentrations of the vitamin in the serum of patients with pernicious anaemia rose from low pretreatment levels to within or above the normal range (Mollin and Ross, 1953).

In this paper we report the length of time that haemopoiesis was normoblastic and the serum vitamin B_{12} concentration was normal in patients with pernicious anaemia given single intramuscular injections of vitamin B_{12}—observations which have a direct bearing on the treatment of pernicious anaemia. In addition, the effect of daily injections of vitamin B_{12} and of treatment with folic acid or folinic acid is reported.

Materials and Methods

Observations are reported on 33 patients with pernicious anaemia in relapse and 13 patients suffering from other megaloblastic anaemias. While in hospital these patients received a diet from which meat was excluded and in which fish, butter, cheese, and eggs were restricted. Patients usually received the vitamin-B_{12}-deficient diet for five to seven days before treatment.

Method of Assay.—The method using *Euglena gracilis* var. *bacillaris* as test organism is based on that introduced by Hutner *et al.* (1949); details of the procedure followed have been described previously (Ross, 1952). Serum for assay purposes was usually taken daily; serial samples taken over a period of one to two weeks were generally assayed in one batch. All the samples were stored frozen and assayed at least twice. The concentrations recorded are total concentrations of vitamin B_{12} equivalent, but, as no significant amount of uncombined vitamin was present in the specimens

*In receipt of a grant from the Medical Research Council.

reported on, the total concentrations in fact also represent combined concentrations. A small number of abnormally high results thought to be due to the presence of some material which is haemopoietically inactive, but able to replace vitamin B_{12} in the growth of the test organism, have been omitted. The occurrence of such apparently non-specific results has been discussed previously (Mollin and Ross, 1952, 1953). Evidence that the Euglena assay is not completely specific for vitamin B_{12} has also been given by Robbins, Hervey, and Stebbins (1952), who found that the organism could grow in the presence of several pure substances which were inactive in the treatment of pernicious anaemia.

Bone-marrow Biopsy.—Serial samples of bone marrow were obtained from the sternum. Approximately 0.2 ml. of marrow was withdrawn into a dry syringe; smears were made immediately from the aspirated material. Air-dried films were stained with May–Grünwald Giemsa.

Serum Vitamin B_{12} Concentrations after Single Injections of the Vitamin

Injections of 20, 40, 80, 160, 320, or 1,000 μg. of crystalline vitamin B_{12} were given to pernicious-anaemia patients. Thirty injections were given to patients who were in relapse and who had received no treatment for at least six months. Seven injections were given to patients who had previously received treatment but in whom the serum vitamin B_{12} concentration had again fallen to low levels and in whom haemopoiesis was once more megaloblastic. The serum vitamin B_{12} concentrations in these patients were measured at frequent intervals, usually daily. Daily blood counts and reticulocyte counts were also made. Marrow biopsies were carried out at frequent intervals in most patients. The amount of vitamin B_{12} excreted in the urine after injections of the vitamin was also measured in most instances. Some of these observations are summarized in Table I; detailed observations on certain of the patients are given below.

Relation Between Serum Vitamin B_{12} Concentration and Haemopoiesis

In Fig. 1 is illustrated an example of the type of study made. This patient (Case 7) was given an initial injection of 80 μg. of vitamin B_{12}, followed 20 days later by an injection of 160 μg. Before treatment the serum vitamin B_{12} concentration was low (55 $\mu\mu$g. per ml.); after the first injection of 80 μg. it remained above the lower limit of the normal range (100 $\mu\mu$g. per ml.) for 11 days. The bone marrow was normoblastic while the serum concentration was normal, but was found to have undergone intermediate megaloblastic change within a few days of the level falling below 100 $\mu\mu$g. per ml. After the injection of 160 μg. the serum vitamin B_{12} concentration remained normal for a further 21 days.

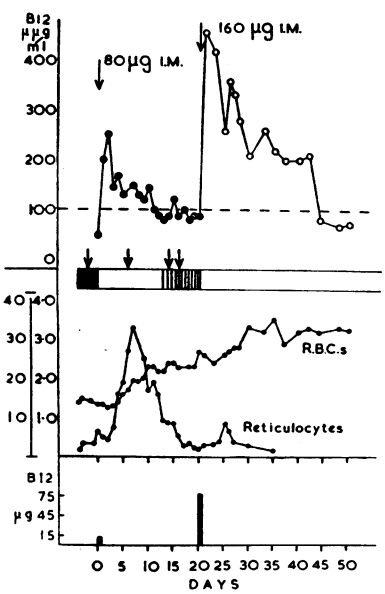

FIG. 1.—Changes in serum and urine vitamin B_{12} concentrations, in bone marrow, and in blood count of a patient with pernicious anaemia after intramuscular injections of 80 μg. and 160 μg. of vitamin B_{12}. (The changes in the marrow are shown diagrammatically in Figs. 1–5. The black area represents megaloblastic haemopoiesis, the vertically lined area represents intermediate megaloblastic haemopoiesis, and the open area represents normoblastic haemopoiesis. The arrows indicate the times of marrow biopsy.)

In Fig. 2 are illustrated examples of the relation between the serum vitamin B_{12} concentration and haemopoiesis in patients with untreated pernicious anaemia following injections of 20 μg. of vitamin B_{12}. Early megaloblastic change was recognized in the bone marrow a few days after the serum vitamin B_{12} concentration fell below 100 $\mu\mu$g. per ml.; at levels greater than this the marrow remained normoblastic.

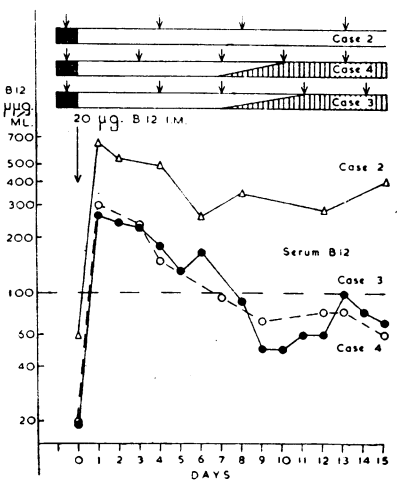

FIG. 2.—Serum vitamin B_{12} concentrations and bone-marrow changes in three patients with pernicious anaemia after intramuscular injections of 20 μg. of vitamin B_{12}.

Similar observations were made on 15 other patients given injections of 20, 40, 80, 160, 320, or 1,000 μg. In all the patients, when the marrows were normoblastic (53 observations) the serum vitamin B_{12} concentrations ranged from 80 to 860 $\mu\mu$g. per ml., with a mean concentration of 250 $\mu\mu$g. per ml.; when they were megaloblastic (28 observations) the concentrations ranged from 50 to 120 $\mu\mu$g. per ml., with a mean concentration of 83 $\mu\mu$g. per ml., except in one patient. This patient, a woman aged 70, who was greatly neglected and before admission had been taking a diet deficient in both folic acid and ascorbic acid, responded well initially to an injection of 160 μg. of vitamin B_{12}, but showed early signs of relapse in the bone marrow when her serum vitamin B_{12} level was still about 200 $\mu\mu$g. per ml.

Duration of Normal Serum Vitamin B_{12} Concentrations after Injections: Relation to Amounts of Vitamin Retained in Body

In Table I is shown the length of time during which the vitamin B_{12} concentrations of the serum of patients with pernicious anaemia remained at or above 100 $\mu\mu$g. per ml. after intramuscular injections of vitamin B_{12}. There was considerable variation in the duration in patients given the same dose of the vitamin, but on the average the concentration remained within the normal range for longer periods after larger injections. In Table I is also shown the amount of vitamin retained after intramuscular injections, calculated by subtracting the amounts of vitamin B_{12} recovered in the urine in the first 24 hours after injections from the amount given. The average time the levels remained within the normal range increased significantly in proportion to the amount of the vitamin retained in the body. However, the differences in duration of normal levels in individual patients could not be entirely explained by differences in retention of the vitamin (see Discussion).

It must be emphasized that the serum vitamin B_{12} levels of patients with pernicious anaemia, although within the normal range for days or weeks after single injections of the vitamin, were usually much lower than the levels found in normal subjects. Even 24 hours after the injections of 40 μg. the mean serum concentration was well below the mean normal level of 322 ± 29 $\mu\mu$g. per ml. (Mollin and Ross, 1952). After injections of 80 or 160 μg. the mean serum concentrations were as high as the mean normal level only for the first four days (see Table II).

TABLE I.—*Duration of Normal Serum Vitamin B_{12} Levels and Normoblastic Haemopoiesis in Patients with Pernicious Anaemia after Intramuscular Injections of Vitamin B_{12}, together with the Amount of the Vitamin Retained*

Case No.	Pre-treatment R.B.C. (mil./c.mm.)	I.M. Dose of Vitamin B_{12} (μg.)	Vitamin B_{12} Retained* (μg.)	Duration of Serum Vitamin B_{12} above 100 $\mu\mu$g. per ml. (Days)	Bone Marrow†	
					Normoblastic (Days)	Megaloblastic (Days)
1	1·6	20	—	11	7	10
4a	1·8	20	—	6	7	10
3	2·0	20	—	7	7	11
2	2·4	20	—	18+	14	—
8	3·1	20	—	7	—	—
4b	3·2	20	—	8	—	8
9	3·3	20	—	8	—	—
10a	1·3	40	34	2	—	3
11	1·5	40	39	8	—	15
12	1·5	40	37	16	14	18
13	2·0	40	37	7	9	11
14	3·2	40	34	22	—	20
15	4·2	40	—	11	—	—
16	1·1	80	58	11	17	21
6	1·2	80	76	27+	23	—
7a	1·3	80	67	11	—	14
17	1·3	80	75	33+	22	—
18	1·3	80	71	24+	3	16
5	2·4	80	71	35+	29	—
19	3·2	80	53	19	—	—
20	3·7	80	63	17	5	—
21	3·7	80	65	32	7	—
22	1·2	160	92	23	24	35
23	1·4	160	—	48	24	49
24	1·5	160	125	29+	26	—
10b	1·6	160	39	11	10	12
7b	2·7	160	72	21	—	—
25a	2·8	160	72	19	—	—
26	3·7	160	97	22	9	—
10c	2·5	320	—	39	25	—
27	2·5	320	124	50+	—	—
25b	4·0	320	255	98	—	—
10d	2·9	1,000	410	51+	—	—
29	2·8	1,000	220	34	7	67
30	3·6	1,000	190	58	—	66
28a	1·9	40 Liver extract	36	17	14	—
28b	2·8	40 Liver extract	37	14+	—	—

* The difference between the dose given and the amount of vitamin found in the urine in the first 24 or 48 hours. In most instances, except with the largest doses, excretion was complete within 24 hours.

† The normoblastic figures are the times of the last marrow biopsy showing normoblastic haemopoiesis. The megaloblastic figures are the times of the first marrow biopsy showing megaloblastic haemopoiesis.

TABLE II.—*Mean Serum Vitamin B_{12} Concentrations in Patients with Pernicious Anaemia Before and After Intramuscular Injections of Vitamin B_{12}*

I.M. Dose of Vitamin B_{12}	Mean Serum Vitamin B_{12} Concentrations ($\mu\mu$g. per ml.)*					
	Pre-treatment†	Post-treatment: Days after Injections				
		1–2	3–4	5–6	7–8	9–10
40 μg.	38±2·2 (60)	181± 29 (11)‡	134± 15 (10)	141±29 (10)	151±41 (9)	94± 8 (8)
80 μg.	40±2·5 (48)	320± 34 (16)	245± 36 (13)	265±33 (12)	207±42 (12)	206±34 (14)
160 μg.	45±3·3 (52)	367± 54 (12)	307± 58 (11)	225±23 (11)	196±22 (12)	165±26 (11)
320 μg.	52±7·2 (8)	698±352 (4)	485±165 (2)	200 (1)	377±76 (2)	360±44 (2)

* Nine pretreatment results (125 to 1,600 $\mu\mu$g. per ml.) and five post-treatment results (460 to 2,000 $\mu\mu$g. per ml.) were excluded because the values found were considered not to represent true serum vitamin B_{12} concentrations.

† The true mean was lower than the stated mean because the values in some sera were too low to be measured.

‡ Number of observations from which the mean is calculated.

Serum Vitamin B_{12} Concentrations after Daily Injections of the Vitamin

In Fig. 3 are compared the effect of daily injections of 1 μg. and that of 40 μg. of vitamin B_{12} given respectively to two patients with pernicious anaemia. The serum vitamin B_{12} concentrations were assayed 24 hours after each injection immediately before the next injection was given. The levels rose to within the normal range 24 hours after the first injection of 40 μg.; they then increased steadily, reaching after 10 days of treatment a level of approximately 1,000 $\mu\mu$g. per ml., this level being maintained as long as the daily injections were given. The vitamin B_{12} concentration of the serum of the patient given 1 μg. daily was not consistently within the normal range—that is, more than 100 $\mu\mu$g. per ml.—until 15 days after treatment started.

Although there was a great difference in the serum vitamin B_{12} levels following these regimes, the differences in the haematological responses were less dramatic. Erythropoiesis in the bone marrow of both patients was normoblastic by the seventh day. The initial red-cell increase was more rapid in the patient given the larger injections, but after the fifteenth day the rate of increase was almost the same.

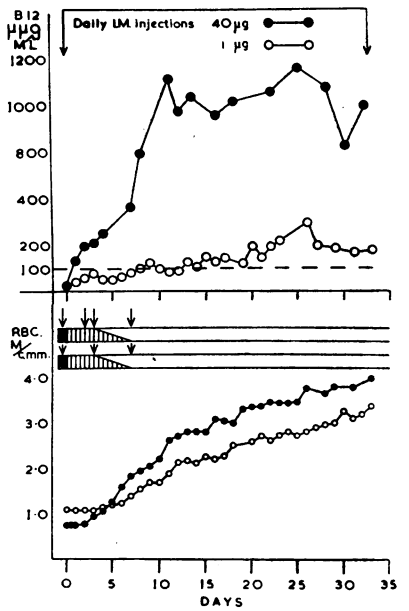

FIG. 3.—Changes in serum vitamin B_{12} concentration, bone marrow, and red-cell count of two patients with pernicious anaemia who received daily injections of vitamin B_{12}.

In another patient given 1,000 μg. daily the serum vitamin B_{12} concentration 24 hours after each injection reached and maintained a level of about 1,300 $\mu\mu$g. per ml. after seven days of treatment.

Serum Vitamin B_{12} Concentrations of Patients Treated with Folic Acid or Folinic Acid

In Megaloblastic Anaemia due to Vitamin B_{12} Deficiency.—The serum vitamin B_{12} concentrations of patients with megaloblastic anaemia due to vitamin B_{12} deficiency remained low when these patients were treated with folic acid by mouth or folinic acid by intramuscular injection.

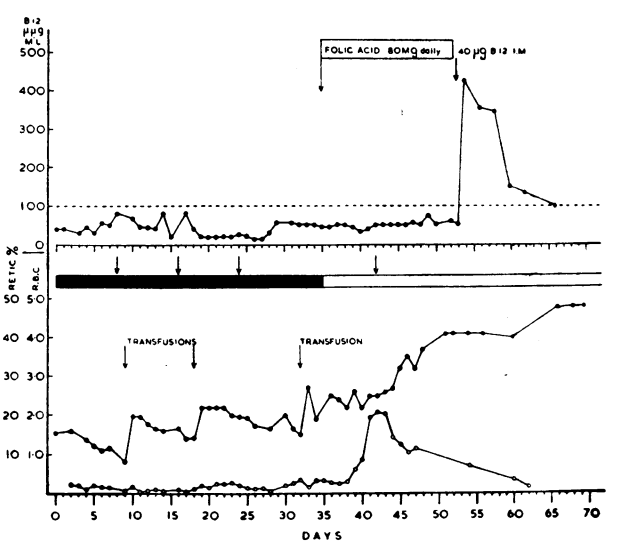

FIG. 4.—Changes in serum vitamin B_{12} concentration, bone marrow, and blood count of a patient with pernicious anaemia (Case 15) after folic acid by mouth followed by vitamin B_{12} by injection.

In Fig. 4 are illustrated the haematological response and the serum vitamin B_{12} concentrations of a patient with pernicious anaemia who was treated first with ineffective preparations of vitamin B_{12} and gastric juice by mouth, then with folic acid and finally with vitamin B_{12}. Similar observations were made on a patient whose megaloblastic anaemia was due to vitamin B_{12} deficiency developing as a result of an ileocolostomy performed to relieve obstruction due to multiple strictures of tuberculous origin. This patient was treated with injections of folinic acid and then with folic acid by mouth.

In Megaloblastic Anaemias due to Deficiency of Folic Acid.—Twelve patients with megaloblastic anaemias associated with the sprue syndrome and/or pregnancy had serum vitamin B_{12} concentrations within the normal range before treatment and failed to respond to treatment with vitamin B_{12}. These patients responded to folic acid by mouth or to folinic acid by intramuscular injection; their serum vitamin B_{12} levels remained normal during their responses to treatment. In Fig. 5 are illustrated the changes in the blood count and serum vitamin B_{12} concentration and bone marrow of one of these patients. She was suffering from steatorrhoea associated with severe pulmonary tuberculosis, and was treated first with vitamin B_{12} and then with folinic acid.

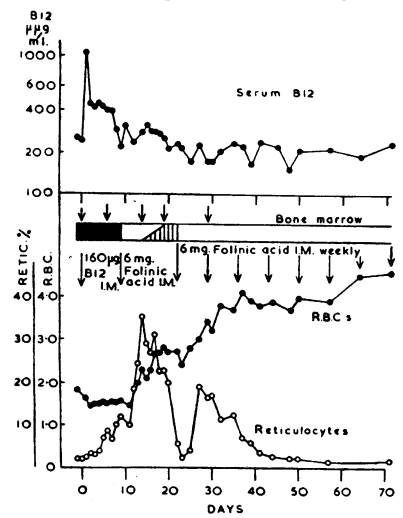

FIG. 5.—Changes in serum vitamin B_{12} concentration, bone marrow, and blood count of a patient with steatorrhoea.

Discussion

Serum Vitamin B_{12} Concentration and Haemopoiesis

In a previous communication (Mollin and Dacie, 1950) it was pointed out that the recognition of the reappearance of early ("intermediate") megaloblastic change in the marrow of patients with pernicious anaemia treated with liver extract or vitamin B_{12} was a sensitive method for determining the time of onset of relapse in these patients. In this paper the changes in haemopoiesis have been correlated with the changes in serum vitamin B_{12} concentration.

While the serum vitamin B_{12} level of patients with uncomplicated pernicious anaemia was within the normal range after injections of the vitamin, haemopoiesis was normoblastic, but when the level fell consistently below the normal range megaloblastic change reappeared in the bone marrow. These findings confirmed our earlier observations that a serum level of around 100 $\mu\mu$g. per ml. was the critical concentration below which signs of vitamin-B_{12} deficiency became recognizable (Mollin and Ross, 1952). However, when very small daily injections of 1 μg. were given to one patient (Fig. 3), the bone marrow was normal by the sixth day, although the serum level 24 hours after each injection was not consistently raised above 100 $\mu\mu$g. per ml. until 15 days after treatment began. Presumably immediately after each injection the serum level became high enough for the marrow to become normoblastic even though there was not enough vitamin B_{12} in the body to keep the serum level above 100 $\mu\mu$g. per ml. throughout the 24 hours after each injection. In fact, it was found in another patient that the serum vitamin B_{12} level rose above 100 $\mu\mu$g. per ml. in the first three hours after a single intramuscular injection of 1 μg. of vitamin B_{12}.

Following the single larger injections of vitamin B_{12} the actual serum level at which megaloblastic change again became recognizable varied. In a few patients the marrow was normoblastic when the serum concentration was as low as 80 $\mu\mu$g. per ml., while in some others the marrow was beginning to show megaloblastic change when the level was as high as 120 $\mu\mu$g. per ml. This is not surprising in view of the probable natural variability in sensitivity of the patients to deficiency of the vitamin and the experimental error of the assay method. Furthermore, the early stages of reversion towards megaloblastic haemopoiesis may not be easy to recognize. This reversion was more pronounced and more easily recognizable in severely anaemic patients given the smaller injections (20 and 40 μg.). In others, signs of reversion were confined to the appearance of a variable proportion of intermediate megaloblasts and abnormal granulocytes. The presence of abnormal granulocytes was useful as an indication of relapse only if leucopoiesis had previously been restored to normal.

Effect of Folic Acid, Folinic Acid, and Vitamin C

The serum vitamin B_{12} levels of the patients with pernicious anaemia in relapse remained low while they were treated with folic acid or folinic acid, though haemopoiesis in their marrow rapidly became normoblastic. The serum levels of patients with megaloblastic anaemia who failed to respond to treatment with vitamin B_{12} were normal before treatment and were not changed by treatment with either folic acid or folinic acid. Therefore the amount of folic acid or folinic acid available for haemopoiesis greatly affects the relation between the serum vitamin B_{12} level and the type of haemopoiesis. The amount of vitamin C available may also affect this relation. In 1942 Dyke, Della Vida, and Delikat claimed that if vitamin C was deficient in the diet of patients with pernicious anaemia the patients might fail to respond to treatment with liver extract. More recently it has been suggested that vitamin C may be concerned in the utilization of folic acid, perhaps by aiding its conversion to folinic acid (Nichol and Welch, 1950; May *et al.*, 1951).

These factors, folic acid and vitamin C, may influence the relation between the serum vitamin B_{12} level and haemopoiesis in two ways. First, if there is severe dietary deficiency of either, megaloblastic haemopoiesis may be present in spite of an adequate vitamin B_{12} level in the serum. A possible example of this has been mentioned earlier in the section on the effect of single injections of vitamin B_{12}. Secondly, if unusually large amounts of folic acid are available, or if it is more efficiently utilized in the presence of ample amounts of vitamin C (Nichol and Welch, 1950), the serum vitamin B_{12} level might be low, though the marrow might be almost normal and the anaemia slight or absent. Patients with pernicious anaemia occasionally have neurological complications but little or no anaemia. The marrows of such patients show early signs of megaloblastic change. The few patients of this type that we have seen have had definitely low serum vitamin B_{12} levels—less than 50 $\mu\mu$g. per ml.—lower than would be expected from the degree of megaloblastic change and anaemia present. It is possible that these patients absorb sufficient folic acid to maintain normal or almost normal haemopoiesis, or utilize the available folic acid more efficiently.

The patients we treated with folic acid did not develop subacute combined degeneration; however, they were treated for short periods only (Fig. 4). If this treatment had been continued, the serum vitamin B_{12} level might have fallen to lower levels with consequent neurological changes.

Duration of Normal Serum Vitamin B_{12} Concentrations after Injections

Though there was considerable variation in the duration of normal vitamin B_{12} levels in different patients given the same dose of vitamin B_{12}, the average duration was longer after the larger injections (Table I). After injections of 20 μg. the serum concentrations fell below the lower limit of the normal range before the tenth day in most patients.

After injections of 40 μg. the level became subnormal before the twenty-second day in all patients, the average duration being 11 days. There was no clear-cut difference in the duration of normal levels after injections of 80 and 160 μg., but some difference would presumably have been demonstrated if larger numbers of patients had been treated. The serum vitamin B_{12} levels of most patients given 80 and 160 μg. were subnormal by the twenty-eighth day, but the serum concentrations of several remained within the normal range for longer than 35 days. The few patients given larger injections (320 and 1,000 μg.) maintained normal serum levels for longer periods. The serum levels of nearly all these patients, although falling below 100 μμg. per ml. in the times stated, were, however, higher than before treatment for at least as long again.

The observations of Walker and Hunter (1952) suggest that when single injections of 1,000 μg. of vitamin B_{12} are given to patients with pernicious anaemia in relapse signs of reversion appear in the marrow of most of them in from 76 to 140 days. In our patients signs of reversion appeared earlier, see Table I.

Injections of Liver Extract.—The duration of normal serum vitamin B_{12} levels in a few patients who received injections of liver extract of known vitamin B_{12} content was similar to that after equivalent doses of crystalline vitamin B_{12} (Table I). We have previously reported that the changes in the serum levels in the first 72 hours after injections of liver extract and of crystalline vitamin B_{12} were similar (Mollin and Ross, 1953).

Relation of Serum Vitamin B_{12} Level to Body Stores of Vitamin B_{12}

It is surprising how effective injections of vitamin B_{12} are in maintaining the serum levels, in view of the extent to which the tissues of patients with pernicious anaemia in relapse are depleted of the vitamin. The observations of Girdwood (1952) suggest that the tissues of normal subjects may contain at least 1,000 to 2,000 μg. of vitamin B_{12} as estimated by the *Lactobacillus leichmannii* assay. He could detect very little or no vitamin B_{12} in the tissues of patients with pernicious anaemia in relapse. Drouet, Wolff, Karlin-Weissman, and Rauber (1951) and Wolff, Drouet, and Karlin-Weissman (1951) found that the average concentration of growth factors for *L. leichmannii* in the livers of patients with pernicious anaemia in relapse was not more than 0.06 μg. per g. This concentration was less than one-tenth of that of control subjects, who may store about 1,000 μg. of vitamin B_{12} in their liver. Single injections of 20 and 40 μg. of vitamin B_{12} obviously could do very little to restore the depleted reserves of vitamin B_{12} in patients with pernicious anaemia in relapse; yet such injections raise the vitamin B_{12} level in the serum to within the normal range for a number of days (see Tables I and II). This suggests that all available vitamin B_{12} in the body is mobilized to maintain a normal serum level as long as possible in order to supply essential requirements; a total of less than 1 μg. throughout the plasma is enough to give a normal concentration (Mollin and Ross, 1953).

Table II shows that really normal levels—that is, 320 ± 29 μμg. per ml.—were maintained for some days only after the larger single injections, such as 320 μg., although injections of as little as 1 μg. daily for 15 days were enough to raise the level to just above the lower limit of the normal range (see Fig. 3).

It would appear, therefore, that the serum vitamin B_{12} level can be maintained within the normal range with very little vitamin B_{12} in store, whereas a much larger amount in store is needed to keep the serum level at the mean normal concentration. Presumably only when the stores of vitamin B_{12} are almost completely exhausted does the serum level fall below the normal range and signs of a megaloblastic anaemia develop.

The tissues of patients in mild relapse, though severely depleted of the vitamin, are presumably less depleted than those of patients in severe relapse. The serum vitamin B_{12} levels of patients with mild relapse, though below 100 μμg. per ml., were rather higher than those of patients with severe relapse (Mollin and Ross, 1952). This may be due to greater stores of the vitamin. We were, however, unable to demonstrate that the duration of normal serum vitamin B_{12} levels was significantly related either to the initial serum level or to the initial red-cell count. However, in certain patients whose pretreatment serum vitamin B_{12} levels were only moderately low—between 60 and 100 μμg. per ml.—the duration of normal levels and the actual height maintained were much greater than the average (see Fig. 2, Case 2).

Implications for Treatment

From the study of bone marrows and serum vitamin B_{12} levels it appears that the average size of injection of vitamin B_{12} required to maintain the marrow normoblastic and the serum level within the normal range, at least during the initial period of the treatment, would be 20 μg. of vitamin B_{12} every 7 days, 40 μg. every 10 days, or 80 to 160 μg. every 15 to 28 days. Some patients require more; others require less. However, it is doubtful if treatment would be adequate if the dose of vitamin B_{12} was restricted to these amounts. At best such doses represent the average amounts required to prevent the appearance of signs of deficiency. It has already been suggested that vitamin-B_{12} deficiency must be extreme before the serum level falls below the normal range and before signs of megaloblastic change appear in the developing haemopoietic cells. Though the blood count usually becomes normal and is maintained at a normal level with the dose regimes mentioned, the tissues probably contain little or no reserve of the vitamin for months or even years after the start of treatment. Nevertheless, the depleted tissue reserves can probably be restored if enough vitamin B_{12} is given during the initial stages of treatment. Drouet *et al.* (1951) found that the concentration of vitamin B_{12} in the liver of patients with pernicious anaemia increased following intramuscular injections of the vitamin, and Girdwood (1952) found vitamin B_{12} in the liver and kidneys of a patient with pernicious anaemia who died shortly after an injection of vitamin B_{12}.

It has already been mentioned that in health the body reserves of vitamin B_{12} may amount to from 1,000 to 2,000 μg. Therefore, if the aim at the beginning of treatment of a patient with pernicious anaemia is to restore to normal the vitamin B_{12} reserve, the total dosage given must ensure the retention in the body of at least this amount. To accomplish this it may be necessary to give a total of as much as 5,000 μg. by injection. Believing it is desirable to replenish the body stores, we found it convenient to give five injections of 1,000 μg. (1 mg.), dissolved in 1 ml. of diluent, in the first week or two of treatment. Of such a dose a large proportion is excreted, but, from the results of assays of the amounts passed in the urine (Chesterman, Cuthbertson, and Pegler, 1951; Mollin and Ross, 1953) and from the long duration of the clinical effect, it would appear that about 200 to 300 μg. of each injection may be retained in the body. Although smaller doses are less wasteful, the more frequent injections required are less convenient. Thereafter injections of 40 to 60 μg. fortnightly or 80 to 100 μg. monthly should be more than enough to maintain the reserves. Large initial doses of liver extract were recommended by Askey (1941) in order to restore the liver reserves of the anti-pernicious-anaemia factor, but the large dosage required can be much more satisfactorily given by means of injections of crystalline vitamin B_{12}.

The observations reported in this paper support the view that the manifestations of pernicious anaemia are primarily due to deficiency of vitamin B_{12}. Conditioned deficiencies of folic acid or ascorbic acid may be present when the deficiency of vitamin B_{12} is severe (Spray, Fourman, and Witts, 1951; Wallerstein, Harris, and Gabuzda, 1952). However, treatment with these substances does not seem to be required provided the patients are taking a good diet and are given adequate amounts of vitamin B_{12}.

Summary

The serum vitamin B_{12} concentrations of patients with pernicious anaemia in relapse rose from low pretreatment levels to within the normal range after single intramuscular injections of 20 to 1,000 μg. of vitamin B_{12}. Concentrations were within the normal range for from 6 to more than 18 days after injections of 20 μg.; from 2 to 22 days after 40 μg.; from 11 to at least 35 days after 80 μg.; from 11 to at least 48 days after 160 μg.; from 39 to 98 days after 320 μg.; and from 34 to at least 58 days after 1,000 μg.

Although the concentrations were within the normal range for these periods, the mean of the concentrations of all the patients in each treatment group fell within a few days of the injections below the mean concentration found in normal subjects.

When the serum vitamin B_{12} concentrations were normal haemopoiesis was normoblastic; when the concentrations were below the normal range megaloblasts reappeared in the marrow.

Intramuscular injections of 40 μg. of vitamin B_{12} every 10 days, or of 160 μg. every 21 days, maintained the serum vitamin B_{12} level of most patients with pernicious anaemia within the normal range. These doses seem to be the minimum requirement for the treatment of most patients with pernicious anaemia in relapse. It is recommended, however, that much larger doses of vitamin B_{12} (up to 5 mg.) should be given in the first week or two of treatment in order not only to supply enough vitamin B_{12} for haemopoiesis but also to replenish the greatly depleted tissue reserves.

The serum vitamin B_{12} concentrations of patients with various megaloblastic anaemias were not altered by treatment with folic acid or folinic acid.

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VITAMIN B_{12} IN NUTRITIONAL MACROCYTIC ANAEMIA

BY

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The efficacy of vitamin B_{12} in pernicious anaemia (P.A.) and its neurological complication is now well established. The exact status of vitamin B_{12} in the treatment of nutritional macrocytic anaemia (N.M.A.) is, however, still uncertain owing to the scarcity of available reports. Folic acid is universally held to be the drug of choice in N.M.A. The present communication deals with trials of vitamin B_{12} in 22 cases of N.M.A. undertaken to find out primarily its value in N.M.A., and incidentally to compare its haematinic efficiency with that of folic acid and/or liver extract.

Materials and Methods

The patients were all Indians. Only uncomplicated cases admitted into hospital were chosen for this study. Vitamin B_{12} was started only when the blood picture had stabilized after admission. In most cases it was administered intramuscularly; in a few cases it was taken orally in tablet form. A course usually lasted for three to five consecutive days. A second and sometimes a third course were given when the improvement was not adequate or sustained—that is, when the blood values either became stationary or dropped after initial improvement. Folic acid was given orally in doses of 30 mg. a day when there was no improvement with vitamin B_{12} or when the improvement was not sustained. Finally, crude liver extract was given in doses of 3 to 4 ml. a day for six to ten days when the improvement with folic acid was found to lag. Ferrous sulphate, 18 to 21 gr. (1.2 to 1.4 g.) a day was given when hypochromicity was noted after treatment with vitamin B_{12}.

During the whole period the patients had an identical diet consisting of 8 oz. (225 g.) rice, 2 oz. (56 g.) peas, 4 oz. (113 g.) potatoes, 8 oz. green vegetables, 4 oz. bread, 2 oz. butter, 2 oz. sugar, 16 oz. (450 g.) milk, 4 oz. fish, 1 lime, 1 banana. This diet, which is relatively poor in animal protein, has been found to have no haemopoietic activity in N.M.A. A high-protein diet has distinct haemopoietic activity in N.M.A. (Das Gupta and Chatterjea, 1951), and it is therefore imperative that in the evaluation of the efficacy of different haematinics the patients be always on a control diet as above.

The degree of improvement after each course of treatment has been calculated according to the formula of Della Vida and Dyke (1942); for working purposes the improvement rate is indicated as follows:

Very good	=	improvement rate	over	90%	
Good	=	,,	,, between	75 and	90%
Very fair	=	,,	,,	50 ,,	74%
Fair	=	,,	,,	30 ,,	49%
Tolerable	=	,,	,,	15 ,,	29%
Nil	=	,,	,, below	15%	