Summary

Of 307 cases of depression given electrical treatment, 69% recovered or were much improved, 28% were improved, and only 3% were unchanged.

Reactive depressions did least well; endogenous depressions with hysterical or obsessional symptoms did less well than other forms. Of endogenous depressions without special features, 81% recovered or were much improved. Involutional depressions, depressions with hypochondriasis, and agitated depressions reacted similarly.

Patients whose recovery was smooth stayed between five and six weeks and averaged 5.4 treatments. Involutional depressions with smooth recovery stayed seven weeks and needed an average of six treatments.

In 26% of all cases the recovery was not smooth or sustained. This "relapse" or "interruption of recovery" is of prognostic significance as regards recurrence.

Of the 81 (26%) cases "relapsing" during treatment, 74 did so between eight and sixteen days after the end of their first course of treatment and only 7 after the sixteenth day—that is, 98% of patients could have been discharged by the sixteenth day after treatment without risk of relapse. Those cases that "relapsed" in hospital needed 2.4 more treatments on an average. There was a special tendency to this form of relapse in the involutional group (46%).

Of 228 patients whose progress was followed over a period of thirteen months from the end of treatment, 23% showed recurrence.

The rate of recurrence was higher in cases of involutional depression, agitated depression, and depression with hypochondriasis (38%, 41%, and 33% respectively). Recurrence bore no relation to the degree of recovery achieved by treatment.

The recurrence rate in all cases is closely related to the "relapse" rate while under treatment, irrespective of the diagnosis. Such recurrence rate is *not* attributable to the frequency in this group of involutional cases, although these happen to have the highest "relapse" rate.

Of those who "relapsed" while in hospital—that is, needed further E.C.T. after a period of seven days or more without it—41% showed recurrence within thirteen months, as opposed to 16% of those with an uninterrupted recovery. This interesting prognostic finding seems not to have been recorded before.

I would like to thank Dr. D. Curran for suggesting this paper, Dr. Curran and Dr. M. Partridge for their help with its composition and for their generously offered suggestions, and to Dr. Fraser Roberts for his advice and for making available his expert statistical knowledge.

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Sir Isaac Pitman and Sons, Ltd., announce that, as from October 1, they have taken over the Lippincott range of medical books. They will now publish these books throughout the world, except in the U.S.A., Canada, Australia, and the Philippines. A new company, Pitman Medical Publishing Co., Ltd., has been formed to take charge of this arrangement, and also to take over Pitman's own medical books.

RAPID ESTIMATION OF THE SERUM VITAMIN B₁₂ LEVEL BY A MICROBIOLOGICAL METHOD

BY

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In a previous investigation attempts to measure the serum level of vitamin B₁₂ by its ability to support the growth of the organism *Lactobacillus leichmannii* were unsatisfactory (Girdwood, 1953a). This was because the diluted, heated serum not only of normal persons but also of those with pernicious anaemia was capable of maintaining the growth of the test organism. It was concluded that this activity was due, not to vitamin B₁₂ itself, but possibly to desoxyribosides of thymine, guanine, and hypoxanthine.

While these observations were awaiting publication, Rosenthal and Sarett (1952) in New Orleans described a method for estimating the serum vitamin B₁₂ level, using *L. leichmannii* as the test organism, and with it they found in normal subjects that the level was of the order of 80–420 µg./ml. The technique adopted by these workers differed in several ways from my own, notably in that the serum was treated with an acetate buffer of pH 4.6. In the earlier investigations I had used either no buffer or a phosphate buffer of pH 6.8. The American workers measured their results by titrating the acid produced by the organisms after incubation for sixty-five to seventy-two hours, whereas I carried out photoelectric measurements of the growth of the organisms after incubation for sixteen hours. Since the apparent serum vitamin B₁₂ levels thus obtained by Rosenthal and Sarett (1952) were much lower than my own figures, it was decided to carry out further investigations.

Most of the data concerning the serum level of vitamin B₁₂ have been derived from measurement of the growth of the protozoon *Euglena gracilis* (Hutner *et al.*, 1949; Ross, 1950; Mollin and Ross, 1953). This method is easy to carry out when the necessary apparatus is available, but its advantage to the clinician is lessened by the fact that results are not available within a week.

The workers at New Orleans have kindly kept us informed of their findings with the *L. leichmannii* assay and have recently published the results of their investigations (Unglaub *et al.*, 1954), which, unlike my own, have concerned the value of the *L. leichmannii* assay in differentiating between the serum of a pernicious anaemia patient and that of other persons. My aim has been to obtain even more rapid readings by incubating the inoculated mixture of serum and culture medium for only sixteen hours.

Material and Methods

Unless otherwise stated, the pernicious anaemia patients were typical cases in relapse. In referring to patients with other forms of megaloblastic anaemia, the term "megaloblastic" is used only where the marrow was similar to that of untreated pernicious anaemia. The controls included approximately equal numbers of non-anaemic persons and of people with non-megaloblastic anaemia.

Into a sterile container was put 20 ml. of blood, which was allowed to clot. The preparation of the serum for

assay was by the method of Rosenthal and Sarett (1952), an acetate buffer at pH 4.6 being used. The medium was modified from that of Thompson *et al.* (1950) by the addition of thioglycollic acid and the withdrawal of ascorbic acid and fumaric acid. It was found that certain batches of hydrolysed casein gave such high blanks that they were unsuitable for use in the medium. The cyanocobalamin standard and the diluted serum were added prior to the autoclaving of the medium, but in our most recent studies we have obtained satisfactory results, with lower blanks, by adding these aseptically. The growth of the test organism, *L. leichmannii*, was estimated by turbidity measurements after incubation for sixteen hours at 37° C.

Results

In Table I are given the results of triplicate assays on the same samples of sera.

TABLE I.—Results of Triplicate Assays of the Serum Level of Vitamin B₁₂ in 10 Patients by *L. leichmannii* Assay

Case	Serum vitamin B ₁₂ (μ g./ml.)		
1	< 50	< 50	< 50
2	< 50	< 50	< 50
3	< 50	< 50	< 80
4	50	80	60
5	100	140	130
6	150	160	160
7	180	280	230
8	185	185	200
9	200	270	240
10	430	450	300

With the kind co-operation of Dr. G. I. M. Ross, of the Postgraduate School of London, specimens of serum were exchanged and estimated in Edinburgh by the *L. leichmannii* technique and in London by the *Euglena* method. The diagnosis was unknown to the recipient in each case, and the results of the assays, which are given in Table II, were posted on the same morning.

TABLE II.—Duplicate Assays of Serum for its Vitamin B₁₂ Content by Two Techniques: Specimens Exchanged Between London and Edinburgh

Case	Diagnosis	Serum vitamin B ₁₂ levels (μg./ml.)	
		<i>L. leitchmannii</i>	<i>E. gracilis</i>
<i>Serum sent from London</i>			
11	Pernicious anaemia in relapse	80	60
12	" " " " " " " "	98	50
13	Pooled sera " " " " " "	310	365
14	" " " " " " " "	370	340
15	" " " " " " " "	490	520
16	Atypical leukaemia	870	1,250
<i>Serum sent from Edinburgh</i>			
17	Subacute combined degeneration	50	50
18	Pernicious anaemia in relapse	125	50
19	" " " " " " " "	120	215
20	" " " " " " " "	150	180
21	" " " " " " " "	300	185
22	Iron-deficiency anaemia	320	195
23	Neurosis	410	380
24	Amoebiasis	440	540

Cases 17 to 21 all responded to vitamin B₁₂ therapy.

Pernicious Anaemia in Relapse.—The number of cases investigated was 36. In 11 of these the reading was less than 50 $\mu\text{g.}/\text{ml.}$ In the others the range was 55–200 $\mu\text{g.}/\text{ml.}$ (mean 95 $\mu\text{g.}/\text{ml.}$). In only two instances did the reading exceed 130 $\mu\text{g.}/\text{ml.}$, and it will be seen from Table II that in one of these a similar high reading was also obtained by Dr. Ross. All the patients responded to treatment with vitamin B₁₂.

Other Forms of Megaloblastic Anaemia.—The results are summarized in Table III. It will be seen that the serum vitamin B₁₂ level was less than 130 $\mu\text{g.}/\text{ml.}$ in one patient with idiopathic steatorrhoea and in one who developed megaloblastic anaemia five years after partial gastrectomy.

TABLE III.—Serum Vitamin B₁₂ Levels (*L. leichmannii*) in Forms of Megaloblastic Anaemia other than Pernicious Anaemia

Cause of Megaloblastic Anaemia	No. of Cases	Mean ($\mu\text{g.}/\text{ml.}$)	Range ($\mu\text{g.}/\text{ml.}$)	No. with less than 130 $\mu\text{g.}/\text{ml.}$
Pregnancy	12	257	160-440	—
Idiopathic steatorrhea ..	10	219	120-370	1
Regional jejunitis ..	1	180	—	—
Partial gastrectomy ..	1	95	—	1

for gastric ulcer. The latter patient was then treated with vitamin B₁₂ alone and showed a good haemopoietic response. It is of interest that another patient who developed macrocytic anaemia seven years after partial gastrectomy for gastric ulcer also had a low vitamin B₁₂ level. The blood findings were Hb, 1.81 g. per 100 ml.; red cells, 2,320,000 per c.mm.; C.I., 1.18; M.C.V. 125 cubic μ . The marrow was not frankly megaloblastic, but transitional erythroblasts were present. A differential urinary folic-acid-excretion test (Girdwood, 1953b) showed no evidence of malabsorption of folic acid. The serum vitamin B₁₂ level was 110 $\mu\mu\text{g.}/\text{ml.}$ It was considered that the anaemia was due to vitamin B₁₂ deficiency, but for experimental purposes folic acid therapy was given alone. After six months of this treatment the serum vitamin B₁₂ level remained unchanged and there was no clinical or haematological deterioration, or any evidence of neurological involvement. In four patients who had *not* developed anaemia after partial gastrectomy the serum vitamin B₁₂ levels were respectively 140, 170, 250, and 250 $\mu\mu\text{g.}/\text{ml.}$

Other Conditions.—In 50 control cases the serum vitamin B₁₂ levels ranged from 50 to 870 $\mu\mu\text{g.}/\text{ml.}$ (mean 320 $\mu\mu\text{g.}/\text{ml.}$). In only three instances was the level less than 130 $\mu\mu\text{g.}/\text{ml.}$ The reading was 50 $\mu\mu\text{g.}/\text{ml.}$ in two patients whose clinical features suggested pernicious anaemia and who had histamine-fast achlorhydria. One of these, who now had macrocytic anaemia and neurological features, had been given forty-eight capsules of a blunderbuss haematinic containing folic acid without vitamin B₁₂ six months previously. In both these patients the marrow was *normoblastic*, but there was a slow haemopoietic response to vitamin B₁₂ therapy. There was no evidence of hepatic or renal disease. In the case of the patient with evidence of subacute combined degeneration of the cord there was sufficient serum for a sample to be sent to Dr. Ross, and with the *Euglena* assay he too estimated the level to be 50 $\mu\mu\text{g.}/\text{ml.}$ The third patient, who had a reading of 70 $\mu\mu\text{g.}/\text{ml.}$, had refractory iron-deficiency anaemia manifesting itself twenty-five years after a gastro-enterostomy operation. The marrow was *normoblastic*.

Pernicious Anaemia under Treatment.—Our findings were similar to those of other workers and need not be given in detail. Serum vitamin B₁₂ levels could be maintained by injections of vitamin B₁₂ or by the oral administration of a preparation of cyanocobalamin and intrinsic factor. In one patient with pernicious anaemia in relapse, 200 µg. of cyanocobalamin snuff daily for fourteen days raised the vitamin B₁₂ level from 90 to 510 µµg./ml.

Discussion

By means of the *L. leichmannii* assay performed in this way it is possible to obtain within twenty-four hours a measurement of the serum vitamin B₁₂ level. The method is speedy, but suffers from the difficulties common to all methods of microbiological assay, and is probably more prone to technical troubles than is the *Euglena* assay. The measurement includes both free and bound vitamin B₁₂.

In most instances the clinician can diagnose pernicious anaemia easily and without complicated biochemical investigations, but in a minority of cases the exact nature of the megaloblastic anaemia may not be clear. In practically all such cases a combination of a serum vitamin B₁₂ assay and

a differential urinary-folic-acid-excretion test (Girdwood, 1953b) will clarify the diagnosis. It should be noted that one cannot exclude malabsorption of folic acid merely by obtaining a normal result with a fat-balance test carried out over a short period of eight days.

Most of the cases reported here with megaloblastic anaemia in association with idiopathic steatorrhoea had a differential urinary-folic-acid-excretion test carried out, and there was evidence of malabsorption of folic acid and a normal level of serum vitamin B₁₂ in all cases except one. These findings explain why most patients with the "malabsorption syndrome" respond so much better to folic acid than to vitamin B₁₂.

In considering serum vitamin B₁₂ levels in pernicious anaemia patients under treatment, it is important to remember that in the normal person there are large stores of cobalamin substances, particularly in the liver (Girdwood, 1952), and that the serum measurement does not necessarily give any information about the extent of depletion of these stores. It is likely that megaloblastic anaemia in pernicious anaemia is a *late* manifestation of generalized deficiency of vitamin B₁₂. I have previously made the suggestion that subacute combined degeneration of the cord may develop in the absence of anaemia because of the presence of folic acid substances in the body, and shown (Girdwood, 1952) that substances with folic acid activity for the growth of micro-organisms may be present in the liver of a pernicious anaemia patient in the absence of vitamin B₁₂. The patient referred to above who had clinical features of subacute combined degeneration of the cord and a low serum vitamin B₁₂ level despite the presence of a normoblastic bone marrow may have had the megaloblastic changes but not the neurological complications prevented by the folic acid in the blunderbuss haematinic that had been given before she was referred to hospital.

Summary

The serum level of vitamin B₁₂ has been estimated by a *Lactobacillus leichmannii* growth method in 36 patients with pernicious anaemia in relapse, 24 patients with other forms of megaloblastic anaemia, 55 persons who did not suffer from megaloblastic anaemia, and 20 patients with pernicious anaemia under treatment.

The level was less than 130 $\mu\mu\text{g./ml.}$ in 34 out of 36 cases of pernicious anaemia in relapse, in one patient who had megaloblastic anaemia and another with macrocytic but not frankly megaloblastic anaemia after partial gastrectomy, in one with idiopathic steatorrhoea, and in three other persons, two of whom had macrocytic anaemia with a normoblastic marrow and who responded to vitamin B₁₂ therapy. It exceeded 130 $\mu\mu\text{g./ml.}$ in all the other patients tested.

The method is speedy, and together with the differential urinary-folic-acid-excretion test is of value in the investigation of the pathogenesis of complex cases of megaloblastic anaemia.

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ANTAGONISTIC EFFECT OF ORAL VITAMIN K₁ ON THE ACTION OF ETHYL BISCOUMACETATE AND PHENYLINDANEDIONE

BY

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There are, to date, few British reports on the value of vitamin K₁ (2-methyl-3-phytyl-1:4:naphthaquinone) for antagonizing or modifying the "hypoprothrombinaemic" effect of the anticoagulant drugs dicoumarol (3,3'-methylene-bis-4-hydroxycoumarin), "tromexan" (ethyl biscoumacetate; bis-3:3'(4-hydroxycoumarinyl)-ethyl acetate), and "dindevan" (phenylindanedione; 2-phenyl-indane-1:3-dione). As the last two drugs are now widely used in the treatment of thrombo-embolic disease, the effect of fat-soluble vitamin K₁ in relation to the use of these anticoagulants requires emphasis.

Material and Methods

A series of experiments was performed on groups of healthy subjects who were taking regular doses of ethyl biscoumacetate or phenylindanedione in therapeutic amounts. At set times, while taking one or other of these

FIG. 1.—Prothrombin activities on stopping tromexan (ethyl biscoumacetate) administration. The arrow indicates when 100 mg. of vitamin K₁ was given orally.