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## COMPARISON OF LIVER EXTRACT AND VITAMIN B<sub>12</sub> (CYANOCOBALAMIN) IN MAINTENANCE TREATMENT OF PERNICIOUS ANAEMIA

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The discovery of vitamin B<sub>12</sub> (cyanocobalamin) (Smith and Parker, 1948; Rickes, Brink, Koniuszy, Wood, and Folkers, 1948), and the investigations which have demonstrated that it is probably identical with Castle's extrinsic factor, have shed further light on the pathogenesis of the megaloblastic anaemias. The effect on medical practice has been less. Indeed, Beckman (1950) says that in the treatment of pernicious anaemia, apart from the fact that it will not cause species sensitization, "it is not superior to parenteral liver extract." We believe that this is not the case and that many physicians and general practitioners have failed to realize the special merits which vitamin B<sub>12</sub> possesses.

It is true that *adequate* treatment with liver extract is perfectly satisfactory. Pernicious anaemia is, however, a disease of variable severity which in its early stages tends to show remissions, and the dose of liver extract required to keep a patient in good health may thus differ greatly from case to case and from time to time. It is the belief of many physicians that liver extracts, at least those which have been available in Great Britain, have fallen in potency since the outbreak of the second world war, and evidence of this has been produced by Mollin (1950). Many patients receiving injections of liver extract from their general practitioners and referred to hospital for blood counts have been found by us to have levels of haemoglobin and red cells well below the optimum. The demonstration (Girdwood, Carmichael, and Woolf, 1950) that the vitamin B<sub>12</sub> content of many samples of liver extract sold in this country is variable and often low lends further support to the view that we have been treating a disease of variable severity with preparations of variable activity. If this is true, the great practical advance in the introduction of vitamin B<sub>12</sub> is the possibility of giving a fixed and indeed a large dose of a pure principle of known high activity. In cases of pernicious anaemia in relapse it has been shown by Ungley (1949, 1951) and others that a single dose of vitamin B<sub>12</sub> of the order of 10 µg. will

usually give a satisfactory reticulocyte response and rise in the red-cell count. Its ability to maintain patients in good health and with a normal blood picture over long periods is less clearly defined.

When vitamin B<sub>12</sub> first became available we were fortunate in having under continuous hospital supervision a moderately large series of patients suffering from pernicious anaemia. The results of treating these and other patients with vitamin B<sub>12</sub> for two years are now reported. The effects of previous treatment with liver extracts were in many cases available for comparison.

### Method and Results

We have now had under our care 60 patients with pernicious anaemia whom we have treated with vitamin B<sub>12</sub>. Our conclusions are, however, based on a detailed analysis of three groups of patients; (1) a group of 22 who had previously received injections of liver extract for relatively long periods and whose progress was then observed for at least two years after a change of treatment to vitamin B<sub>12</sub> injections; (2) two groups each consisting of 10 patients who had received either liver extract alone or vitamin B<sub>12</sub> alone from the onset of the illness. In the remaining 28 patients treated with vitamin B<sub>12</sub> the periods of observation were shorter or else the haematological observations had been made at too long intervals to allow of satisfactory statistical analysis. In these, however, the response to treatment was similar to that observed in the more fully investigated groups, and nothing was noted in them that would lead us to modify our general conclusions in any way.

The 22 patients in the first group had attended the clinic regularly and had had blood counts carried out at approximately three-monthly intervals. Most had received their injections of liver extract at the clinic, and all were seen and questioned about their progress at each visit. All were patients who were known to have had pernicious

anaemia for relatively long periods. Vitamin B<sub>12</sub> was given to these cases and blood counts and clinical examinations were carried out every six to eight weeks. Ten micrograms of vitamin B<sub>12</sub> was initially substituted for each 1 ml. of a liver preparation, and injections were given at the same relative times. Later the period between injections was lengthened so that some patients were receiving injections only once a month. They have now all been treated with vitamin B<sub>12</sub> for at least two years.

The results in these 22 patients are shown in Table I. The mean haemoglobin and the red-cell count have been calculated for the period on liver treatment, which

TABLE I.—A Comparison Between the Average Haemoglobin Concentrations and Red-cell Counts in Patients on Maintenance Treatment with Liver Extracts and for at Least Two Years on Vitamin B<sub>12</sub>

Case No.	Liver Extract				Vitamin B <sub>12</sub>				
	Preparation	Injection (ml./month)	Av. Hb % (100% = 14.8 g.)	Av. R.B.C. (mill./c.mm.)	µg. Vitamin B <sub>12</sub> per month	Av. Hb % (100% = 14.8 g.)		Av. R.B.C. (mill./c.mm.)	
						A	B	A	B
1	C	32	81	3.97	160	91	87	4.49	4.27
2	B	24	86	4.00	80	93	93	4.51	4.46
3	A	16	101	4.40	40	104	104	5.12	5.05
4	C	48	77	3.88	160	84	82	4.07	3.86
5	B	4	87	4.24	80	94	93	4.40	4.46
6	A	32	95	4.45	80	104	104	5.07	5.95
7	C	8	95	4.67	160	101	100	4.90	4.93
8	C	32	92	4.72	160	94	93	4.68	4.65
9	B	16	85	4.26	160	96	91	4.68	4.63
10	B	8	100	4.73	80	95	95	4.70	4.65
11	B	16	95	4.15	160	96	98	4.52	4.65
12	A	4	94	4.30	40	97	96	4.58	4.65
13	C	4	94	4.52	40	97	97	4.68	4.71
14	A	16	83	4.17	80	88	90	4.41	4.48
15	B	8	90	4.19	80	95	96	4.67	4.57
16	C	24	83	4.03	160	91	93	4.27	4.39
17	B	16	91	4.34	80	104	101	4.94	4.89
18	C	12	90	4.48	80	90	90	4.54	4.56
19	C	32	89	4.12	160	97	94	4.35	4.42
20	B	8	88	4.41	80	90	87	4.43	4.41
21	C	4	92	4.58	40	90	93	4.08	4.09
22	B	16	95	4.60	50	98	95	4.74	4.77

The statistical analysis was not carried out on these average figures, but on a consideration of all the available data for each patient. The vitamin B<sub>12</sub> was given originally in a concentration of 20 µg. per ml., so that no patient ever received more than 8 ml. a month. This dose can now be given in 1.6 ml. For a description of columns A and B, see text.

ranged between 8 months and 6 years (average 34 months). The mean values for the first 18 months on vitamin B<sub>12</sub> appear in column A and those including the subsequent six-monthly period in column B. An examination of these figures shows that in 17 out of the 22 patients the mean values have increased while on vitamin B<sub>12</sub> therapy. These results might be regarded as suggesting an improvement brought about by the change to vitamin B<sub>12</sub>. We are, however, fully aware of the errors inherent in haematological observations. These were reduced as much as possible by the estimations being carried out in one department, using the same standard methods throughout. Nevertheless there was considerable variability in the observations in each patient while on either form of treatment, and a reliable comparison cannot be made by use of the means alone. A more detailed statistical analysis has accordingly been carried out on the individual results from which the means in column B (Table I) were calculated, in order to take this variability into account. In this way it was shown that vitamin B<sub>12</sub> gave a significantly better result than liver extract in both haemoglobin and red-cell counts. This difference was obtained by testing the "between treatments within patients" variance against the "within treatments" variance in Table II. The variance ratio was significant at the 1% level of probability (Fisher and Yates, 1948).

TABLE II.—Showing Results of Analysis of Variance of Data from Cases 1-22

Source of Variation	Haemoglobin Estimations			Red-cell Counts		
	Variance	Degrees of Freedom	Variance Ratio	Variance	Degrees of Freedom	Variance Ratio
Between patients	458.7	21		1.03	21	
Between treatments within patients	121.4	22	2.23	0.1777	22	2.07
Within treatments	54.2	402		0.0856	275	
Total	..	445			318	

The difference between treatments for individual cases was tested against a standard error based on the "within treatments" variance. This is the best estimate of the standard error. Vitamin B<sub>12</sub> gave significantly better results in five cases (Nos. 1, 14, 15, 16, and 17) for the haemoglobin, and for eleven cases (Nos. 1, 2, 3, 6, 9, 11, 12, 14, 15, 16, and 17) for the red-cell counts. Only one case showed while receiving vitamin B<sub>12</sub> a decrease in both estimations, and this was not significant. Case 21 showed a significant decrease in the red-cell count only. In the remaining cases the number of blood estimations was smaller and the slight increase in the mean values while on vitamin B<sub>12</sub> therapy was not significant at the 5% level of probability.

Many of the means in column B of Table I, which are calculated from the results obtained during approximately two years of vitamin B<sub>12</sub> therapy, are lower than those in column A, which are calculated from the results obtained during the first eighteen months. Statistical analysis shows that for haemoglobin the aggregate effect is a significant decrease (at the 1% probability level) between the two sets of means. There is, however, no significant difference in the red-cell counts. Haemoglobin levels are of course influenced by factors other than the presence of an adequate amount of liver principle.

A study of the individual cases showed that the first few counts were often higher after the change from liver to vitamin B<sub>12</sub> treatment, and these figures might have produced the significant increases found in the variance analysis. We therefore compared the results of treating ten patients with pernicious anaemia who received vitamin B<sub>12</sub> from the start of their illness for periods ranging from six months to two years (Cases 33 to 42 in Table III) with a group of ten patients who had received liver extract for a similar period (Cases 23 to 32 in Table III). The average maintenance dose

TABLE III.—Haemoglobin Concentration and Red-cell Counts in 10 Patients Maintained on Liver Extracts and on 10 Patients Maintained on Vitamin B<sub>12</sub> in a Dose of 50 µg. a Fortnight or Occasionally 100 µg. a Month for Six Months to Two Years

Case No.	Patients Treated with Liver Extract Only		Case No.	Patients Treated with Vitamin B <sub>12</sub> Only	
	Average Hb % (100% = 14.8 g.)	Average R.B.C. (mill./c.mm.)		Average Hb % (100% = 14.8 g.)	Average R.B.C. (mill./c.mm.)
23	86	4.18	33	92	4.59
24	85	4.01	34	91	4.53
25	97	4.71	35	98	4.66
26	86	4.21	36	96	4.50
27	95	4.40	37	88	4.12
28	93	4.27	38	106	5.13
29	91	4.47	39	99	4.93
30	104	5.15	40	98	4.83
31	94	4.20	41	98	4.53
32	90	4.38	42	93	4.70
Grand mean	92	4.40		96	4.65

was 50 µg. a fortnight although some patients were given 100 µg. every month. The figures analysed were those obtained after complete clinical and haematological response. The result in each of these cases was satisfactory (Table III).

An analysis of variance again showed that vitamin B<sub>12</sub> gave significantly better results than liver extracts. The difference which was significant at the 1% level of probability (Fisher and Yates, 1948) was obtained by testing the "between treatments" variance against the "residual error" variance in Table IV. Inspection of the figures showed no tendency to fall off with time, and the patients remained well on both treatments.

TABLE IV.—Showing Results of Analysis of Variance of Data from Cases 23–42

Source of Variation	Haemoglobin Estimations			Red-cell Counts		
	Variance	Degrees of Freedom	Variance Ratio	Variance	Degrees of Freedom	Variance Ratio
Between treatments	998.0	1	16.4	1.71	1	8.6
Between patients within treatments	225.5	8		0.588	8	
Residual error	60.9	39		0.198	102	
Total		158			104	

#### Other Haematological Observations

White-cell counts were estimated, and prothrombin levels were determined, using a slightly modified Quick one-stage method, in all the patients having maintenance treatment on vitamin B<sub>12</sub> and in 17 patients receiving liver extracts. The leucocyte counts were all within the normal range. Two patients receiving vitamin B<sub>12</sub> had prothrombin levels slightly below the average normal level but still within the normal range. Neither showed any clinical manifestation of prothrombin deficiency.

With one exception all the patients treated with vitamin B<sub>12</sub> remained well clinically. No instances of sore tongue or of gastro-intestinal disturbance were seen. Symptoms or signs of neurological involvement did not appear. Patients said they felt well, and none wished to revert to liver therapy.

The following are some case histories of patients who were strikingly improved by the change from liver extract to vitamin B<sub>12</sub>.

A man aged 32 was diagnosed by his own practitioner as a case of pernicious anaemia in February, 1948, and was treated with liver extract A in doses of 1 ml. weekly for seven months, followed by extract B in doses of 1 ml. weekly for four months, then by extract A at first in a dose of 2 ml. every 14 days and then every week. After a further nine months he still felt ill, and was referred to hospital for the first time. A blood count showed Hb 80% (11.4 g.%) and red cells 2,500,000 per c.mm. His bone marrow was megaloblastic. He was placed on 40 µg. of vitamin B<sub>12</sub> every 14 days, and has since felt well and maintained a normal blood picture.

A woman aged 58 was a known case of pernicious anaemia who before we saw her had been treated with 2 ml. of liver extract C weekly for six months, followed by 2 ml. of extract B for a further eight months. At the end of this time her marrow was megaloblastic and her peripheral blood count showed Hb 50% (7.4 g.%) and red cells 2,020,000 per c.mm. She felt ill. On 40 µg. of vitamin B<sub>12</sub> every three weeks the blood count has remained normal for the past 18 months and she has felt very well.

A woman aged 63 was treated with liver extracts from April, 1946, to December, 1950. On a dose of 4 ml. weekly her blood count was only Hb 85% (12.3 g.%), red cells 3,800,000 per c.mm. She has now received 40 µg. of vitamin B<sub>12</sub> fortnightly for one year, and the haemoglobin has risen and remained above 90% and the red cells have increased to 4,500,000 per c.mm. She feels better.

Only one patient has had a relapse while on vitamin B<sub>12</sub> therapy. She was a woman aged 59 who had received 4 ml. of a liver preparation weekly for 19 months. She had

been given this high dose because she had shown evidence of subacute combined degeneration of the cord at her first visit. She remained well on 40 µg. of vitamin B<sub>12</sub> a fortnight for four months, but when the interval between injections was extended to one month, although the dose was 100 µg., she complained of paraesthesiae and developed some ataxia. The blood count had fallen. Symptoms subsided rapidly and the blood count rose when 50 µg. of vitamin B<sub>12</sub> was given weekly.

#### Discussion

It is generally agreed that injections of vitamin B<sub>12</sub> will give a satisfactory haematological response and a clinical remission when injected into patients with pernicious anaemia in relapse. Several observers have, however, produced evidence suggesting that in long-term treatment liver extracts have advantages. Thus Meacham, Vignos, Heinle, Weisberger, and Epstein (1950) found that 13 out of 30 patients treated for between 5 and 14 months with vitamin B<sub>12</sub> had a fall in red-cell count of over 500,000 per c.mm. They suggested that their results could be explained if liver extracts contained, in addition to vitamin B<sub>12</sub>, other accessory factors necessary to the maintenance of normal haemopoiesis. They thought it more probable, however, that the dose which they were giving was inadequate. The preparations they used were concentrates assayed biologically, and thus did not necessarily contain the full stated content of pure crystalline vitamin B<sub>12</sub>. Since the dose was also low, only 30 µg. a month, it is perhaps not surprising that full effects were not obtained. Similar results to which the same criticisms apply were reported by Beard, McIlvanie, and Nataro (1950).

At the Third International Congress of Haematology, held in Cambridge in August, 1950, many speakers voiced doubts about the capacity of vitamin B<sub>12</sub> to replace liver extracts completely. Thus Goldsmith (1951) found a gradual fall in the erythrocyte count of two patients with pernicious anaemia on vitamin B<sub>12</sub> therapy over a period of several months. Increasing the dose of vitamin B<sub>12</sub> and decreasing the interval between injections did not influence the findings in either of these patients over the next two months. McSorley (1950) said that vitamin B<sub>12</sub> therapy left the leucopenia unaffected in four cases. Beard (1950) transferred 37 cases from liver extract to vitamin B<sub>12</sub> in a dose of 30 µg. per month with immediate improvement, but the blood count fell after 7–11 months, although the dose was raised to 100 µg. a month. Wilkinson (1950) reported glossitis and poor counts on 20–60 µg. weekly.

Other observers have reported satisfactory results on vitamin B<sub>12</sub> treatment. Kinnear and Hunter (1950) placed a series of 20 cases, after initial treatment with liver extract, on 20 µg. of vitamin B<sub>12</sub> every three weeks. In all but one case, which was complicated by haemorrhage, a satisfactory blood picture was maintained for six months. Mills and Hemsted (1951) substituted vitamin B<sub>12</sub> for liver extract in the treatment of all their cases of pernicious anaemia: 37 cases received 20 µg., 49 received 40 µg., and 58 received 80 µg. of vitamin B<sub>12</sub> each month by intramuscular injection for a period of one year. The higher the dose the higher was the red-cell count in general. In 65% of cases the level of the latter was distinctly higher than during liver maintenance therapy, 32% showed no significant difference, and only 3% were worse. Ungley (1951) found that a dose of 10 µg. of vitamin B<sub>12</sub> every two weeks was effective in most of a group of 19 patients for periods up to three years. In the exceptions there were transient symptoms, such as sore tongue, which disappeared spontaneously or were abolished by increasing the dose. He rightly states that such low maintenance doses would be dangerous for patients not under close observation.

It has been shown that certain clinically unimportant abnormalities in the blood picture tend to persist on treatment with purified liver extracts or vitamin B<sub>12</sub> but to be rectified by the use of crude liver extracts or proteolysed liver. These include macrocytosis associated with the presence of a foetal haemoglobin and an abnormal pro-

thrombin content of the blood (Owren, 1950, 1951; Larsen, 1951). Owren (1950) has postulated a "protein synthesis liver factor" which is itself haematologically inert but which is able to correct the above abnormalities. In our series of cases we could not demonstrate the presence of a low prothrombin content of the blood, using a slightly modified Quick one-stage technique, which was less sensitive, however, than the technique used by Owren (1949). Since many thousands of patients have now remained perfectly well for very many years on treatment with purified liver extracts alone we cannot feel that this protein synthesis factor has any practical significance in the treatment of pernicious anaemia.

Nine of our patients were selected at random. Of the eight patients on vitamin B<sub>12</sub> therapy, six showed degrees of macrocytosis varying between  $4.5\% \pm 3.2$  (2 S.E.) and  $22.5\% \pm 5.8$  as judged by Price-Jones curves, while the single patient on purified liver extract treatment showed a macrocytosis of  $27\% \pm 6.2$ .

In our cases, apart from the one case (No. 21) mentioned above, over a period of two years, vitamin B<sub>12</sub> always maintained the red-cell count at the same or higher levels than liver extract had done in the same patients. Moreover, the result was achieved with a much smaller dose of vitamin B<sub>12</sub> in terms of millilitres of injection. This is a point of some practical importance to a patient, since bulky injections are painful. We saw no example of glossitis or persistent leucopenia.

Mollin and Dacie (1950) found that to maintain a normoblastic bone marrow for 15 days it was necessary to give doses of the order of 30–40 µg. of vitamin B<sub>12</sub> or of a liver extract shown by assay to contain this quantity of vitamin B<sub>12</sub>. Half this quantity would give "average satisfactory" responses in the majority of patients, but the marrow was megaloblastic before the end of the 15-day period. On our dosage scheme we saw only the one case of clinical and haematological relapse which we have already described in detail, and this was clearly an instance of too infrequent dosage. In view of Mollin and Dacie's results, and on our own experience, we now advise a dose of 50 µg. of vitamin B<sub>12</sub> given every fortnight, except that we give 100 µg. weekly in cases in which there are neurological complications.

We are aware that our series is relatively small and that studies on the effect of treatment with vitamin B<sub>12</sub> carried out on much larger numbers of patients for longer periods will be reported from other centres. Comparisons with simple liver extracts may, however, prove to be more and more difficult if manufacturers fortify their products with added vitamin B<sub>12</sub>; many are now assaying their extracts biologically and claiming a relatively high content in terms of vitamin B<sub>12</sub> activity. Wide variations in potency and cost will probably remain.

We feel we can confidently recommend physicians and general practitioners to change their patients' treatment from liver extract to vitamin B<sub>12</sub>, and this recommendation would have been the same if our patients' blood pictures had remained unaltered by the change to vitamin B<sub>12</sub> instead of showing a slight overall improvement. We feel sure that on vitamin B<sub>12</sub> treatment for at least two years a person with pernicious anaemia will remain clinically and haematologically as well as or better than he now is on liver therapy. He will gain because he will have a much smaller bulk of injection and he will be certain of receiving an effective dose. The community of which he is a member will save money, too; for the price of a minimum monthly effective dose of liver extract (4 ml.) is about five times that of a certainly effective dose of vitamin B<sub>12</sub> (100 µg.). In our series (Table I), in which the average dose of liver extract was very high, the cost has been reduced to one-twentieth by changing to vitamin B<sub>12</sub> injections. There seems, indeed, little justification for the continuance of a relatively expensive form of treatment unless better evidence than that now available can be brought forward to support it.

### Summary

Sixty patients suffering from pernicious anaemia have been maintained in good health on injections of vitamin B<sub>12</sub> (cyanocobalamin).

Of these, 22 had been previously treated with liver extracts for relatively long periods and were then given vitamin B<sub>12</sub> for at least two years. Only one case showed a statistically significant decrease in the red-cell count; 11 cases had a significant increase. No case showed a significant decrease in haemoglobin concentration.

The first 10 of the patients who had been treated with vitamin B<sub>12</sub> from the start of their illness showed significantly higher red-cell counts and haemoglobin concentrations when compared with 10 patients who received liver extract alone.

A few patients were strikingly better on vitamin B<sub>12</sub> treatment; one relapsed because of too infrequent dosage.

No evidence was found of sore tongue, gastrointestinal disturbance, neurological involvement, or leucopenia. Macrocytosis may persist.

Vitamin B<sub>12</sub> injections are small in bulk, constant in potency, relatively cheap, and do not lead to sensitization. A maintenance dose of at least 50 µg. a fortnight is recommended for all patients with pernicious anaemia without neurological involvement.

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In the House of Commons last week Mr. Frederick Willey raised an interesting point when he asked the Minister of Food what is known about the consumption of rationed and otherwise controlled foods by the various social groups in this country. Major Lloyd George told him he thought the most accurate data available were those provided by the sample inquiries made under the National Food Survey for the quarter ended March 31. According to this all social groups took up their full rations except for bacon. Analysis of the consumption of individual foods showed that the highest social group took up less of their bacon ration than the lowest; the former bought 93% of the total entitlement, and the latter as much as 98%. In other words, the poor eat more bacon than the rich. In the case of eggs, however, the opposite happens. People in the lowest social group ate on the average three eggs each per week. Those in the highest, however, managed to eat three and three-quarter eggs. As for milk, the lowest group used just over four and a half pints each week, and the highest six.