THE VITAMIN B_{12} CONCENTRATION IN LIVER EXTRACTS AND A NOTE ON THE RELATIONSHIP BETWEEN CLINICAL RESPONSE AND B_{12} DOSAGE

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Received July 12, 1949

Vitamin B_{12} Content of Liver Extracts

A number of different liver extracts have been assayed for vitamin B_{12} activity by the cup-plate method of Cuthbertson[1] employing *Lactobacillus lactis* ATCC 8,000. Some of these samples have also been assayed by the technique of Lees and Emery[2] employing the tube assay with *Lactobacillus leichmannii* 313. The results obtained are summarised in Table I.

TABLE I

B_{12} CONTENT OF LIVER EXTRACTS FOR PARENTERAL USE

Distributor	Manufacturer	Extract	μg. B_{12}/ml.‡	Potency
British	A	1	1·4	low
		2	5·0	high
	B	3	6·0	high
	C	4	2·9*	high
		5	0·8	low
	D	6	1·0*	medium
		7	12*	high
	E	8	3*	low
		9	12*	high
	F	10	0·2	high
European	G	11	(*a*) 6*	low
			(*b*) 3·4	low
			0·23	low
	H	12	2·5*	high
	I	13	0·5	high
		14		
	J	15	0·1	medium
		16	0·1	high
	K	17	0·1 μg. B_{12} <0·05†	medium high
American	L	18	2·7	low
	M	19	3·5	medium
	N	20	22	high
	O	21	1·8	high
	P	22	11·8	high

* Average of 2 to 8 different batches. † No B_{12} detected even after chromatography.
‡ The essay results given in Tables I, III, IV and V represent the activity of one or more of the vitamin B_{12} group of factors expressed as the concentration of standard vitamin B_{12} (μg/ml) giving the same response as the sample.

Samples used for assay are all recent, having been obtained for the most part in June, 1949, except for samples from manufacturer G of

which (a) were obtained during 1936 to 1939 and (b) was captured during the war. Of the above samples N, O and P are known to have been manufactured in America. The other samples whose manufacturers are described as European or American were nevertheless presumably made in England, since we are informed that no liver extracts have been imported into this country for the last 6 years. The description used is thus the country of the manufacturers' headquarters, but not necessarily of the extract's origin.

The extracts have been classified into groups of low, medium and high potency on the basis of information supplied with the extracts by their manufacturers. This classification, shown in Table II, is necessarily very rough in the absence of any standard method of describing potency, but in assigning the extracts to low, medium or high potency groups, the suggested dosing schedules, U.S.P. units and liver equivalents have been taken into consideration.

TABLE II

Potency	Stated liver equivalent ml.	U.S.P. units
Low	<10 g.	2
Medium	10 to 20 g.	10
High...	>20 g.	15

No attempt has been made to distinguish between highly refined and highly concentrated extracts, although it is obvious that some manufacturers have attempted to produce potent refined materials, while others appear merely to have concentrated their liver extracts without any great degree of purification.

Table I demonstrates the wide differences between extracts from different manufacturers, the low potency extracts ranging from 0·23 to 6 μg./ml., while medium and high potency extracts vary over the ranges of 0·1 to 3·6 μg. and 0·1 to 22 μg. B_{12}/ml. respectively. These variations between samples of different origins would hardly be expected if adequate clinical trials had been carried out on all batches. This variation may be

TABLE III

BATCH TO BATCH VARIATION OF SAME BRAND OF EXTRACTS FROM DIFFERENT MANUFACTURERS

Manufacturer	Extract	Sample	μg. B_{12}/ml.	Manufacturer	Extract	Sample	μg. B_{12}/ml.
X	A	1	4·8	Z	C	12	15·0
		2	19·8			13	7·5
		3	11·3			14	10·0
Y	B	4	2·7			15	8·0
		5	3·6			16	5·0
		6	2·1			17	5·0
		7	3·0			18	15·0
		8	3·3			19	9·0
Z	C	9	24·0			20	17·5
		10	6·0			21	12·0
		11	9·0			22	12·0

ascribed to differences in manufacturing technique, quality of raw materials and clinical control (if any) of the products. Different batches of the same extract made by the same manufacturer may show a wide range of B_{12} concentrations, but it cannot be said whether these may not be due to alterations in procedure and materials available for manufacture. Table III shows the degree of variation encountered.

From the results it is clear that little value can be attached to estimates of potency unless better control is used than apparently at present. A number of these samples have been assayed by both of the different techniques employed in these laboratories, with the results shown in Table IV.

TABLE IV

MICROBIOLOGICAL ASSAY OF PARENTERAL LIVER EXTRACTS BY CUP-PLATE (*L. LACTIS*) AND TUBE (*L. LEICHMANNII*) METHODS

Extract	Tube assay μg. B_{12}/ml.	Plate assay μg. B_{12}/ml.
1	0·05*	<0·1*
2	0·04*	<0·05*
3	0·6*	<0·1*
4	ca 0·1*	<0·2*
5	0·14	0·22
6	0·4	0·8
7	0·7	1·0
8	1·5	1·4
9	2·8	2·7
10	3·0	3·5
11	3·3	3·3
12	9·7	10·5

* High concentrations of desoxyribosides present in these samples.

On the whole the agreement is reasonable for methods involving two different organisms and two different techniques (the cup-plate and tube method) having different sensitivities to interfering substances. The discrepancies encountered are being further investigated.

RELATIONSHIP BETWEEN CLINICAL RESPONSE AND MICROBIOLOGICAL ACTIVITY

All liver extracts prepared in these laboratories are clinically tested before they are released for sale. For this purpose typical cases of Addisonian pernicious anæmia in severe relapse are used, preferably those showing a red cell count between 1 and 2 million/cmm. The patients receive, by intramuscular injection, a single test dose of the extract. During the following 14 days the blood picture is determined on alternate days, but daily observations are made at the time when the peak of the reticulocyte response is expected. In interpreting the results particular attention is paid to the red cell response, which should increase at the rate expressed by the formula of $I = 0{\cdot}94 - 0{\cdot}214\ E_0$ (Della Vida and Dyke[3]), where I is the weekly increment in the red cells and E_0 is the initial cell count. In assessing the response of an individual patient, consideration is given to other factors that may bear on the test, e.g., hæmoglobin levels, shape of the response curves, reticu-

locyte response, infection and possible iron deficiency or abnormal red cell destruction. Foods that might have an anti-anæmic action are withheld before and during the period of test.

The results on 14 different liver extracts with 18 patients in a number of different hospitals are summarised in Table V, which gives only the red cell response as a percentage of that to be expected from the Della Vida and Dyke formula and the B_{12} content of the different extracts. The other observations made on these patients have been omitted for clarity.

If the responses of the individual patients are considered and if a response of 90 per cent. of that expected is taken as indicating activity of the liver extract, then it can be seen that samples containing less than

TABLE V

RELATION BETWEEN CLINICAL RESPONSE AND B_{12} ACTIVITY OF EXTRACT (1 ML. OF EXTRACT USED FOR THESE TESTS)

Sample	B_{12} activity μg./ml.	Increase in red blood cells expressed as percentage of expected response	Sample	B_{12} activity μg./ml.	Increase in red blood cells expressed as percentage of expected response
1	2·0	75	9	9·0	62
2	2·9	nil, 114 } 57	10	9·0	58
3	3·0	nil	11	12·0	46, 145 } 91
4	4·0	76	12	12·0	73, 138 } 106
5	4·1	nil			
6	4·5	54	13	15·0	93
7	6·0	50	14	17·5	96, 112 } 104
8	6·0	109			

10 μg./ml. are much less satisfactory than those containing more than 10 μg./ml. Of the 11 patients receiving less than 10 μg. of B_{12} 9 gave unsatisfactory responses, while of the 7 patients who received more than 10 μg. only 2 gave unsatisfactory responses. Several of these extracts were tested on more than one patient. The marked variation in response from patient to patient is very clearly seen in the results obtained with these. In particular the different patients receiving samples 2 and 11 show very wide differences in response. If the results obtained with the two patients on each sample are averaged, then it can be seen that only one extract out of 10 containing less than 10 μg./ml. would satisfy our criterion (and this result depends on the reaction of only one patient) while none of the four extracts containing more than 10 μg. B_{12}/ml. would have failed to do so.

SUMMARY

1. The vitamin B_{12} contents of a number of liver extracts have been reported.

2. Assay results using *Lactobaccillus lactis* and *L. leichmannii* have been compared.

3. The clinical responses to a number of extracts have been compared with their B_{12} contents, and the range of activity found by clinical test has been indicated.

We wish to express our thanks to the pathologists without whose enthusiastic co-operation the standardisation of liver extracts would not have been possible.

REFERENCES

1. Cuthbertson, *Biochem. J.*, 1949, **44**, v.
2. Lees and Emery, *Biochem. J.*, 1949, **45**, ii.
3. Della Vida and Dyke, *Lancet*, 1942, **243**, 275.

DISCUSSION

THE three papers dealing with the micro-biological assay of liver extracts by Mr. Shaw and by Dr. Cuthbertson, Miss Lloyd, Dr. Emery and Mr. Lees were discussed together; the last paper was read by Mr. Lees.

MR. SHAW, in presenting his papers, stated that since submitting them he had found that on applying his assay to a commercial solution of crystalline vitamin B_{12} prepared for injection, the indicated vitamin B_{12} content appeared to be approximately three times as great as the labelled value, using as reference standard the solid liver preparation supplied by Dr. Rickes and standardised by him at 0·4μg. per mg. This observation indicated that the results quoted in the paper represented not necessarily vitamin B_{12} as such but were a measure of the growth activity for *Lactobacillus lactis* Dorner. This discrepancy along with the extremely slow speed of migration of the main constituent on paper chromatography, and the American view that microbiological assay of liver extracts for vitamin B_{12} is not reliable unless the vitamin B_{12} content of the liver solids in the preparation under test is of the order of 50 per cent. suggests the possibility that the clinical action of liver extracts may be due to a complex or conjugate of vitamin B_{12} more than to the presence of the free vitamin. It might well be that for the assay of liver extracts a standard liver preparation will be a more satisfactory reference standard than pure crystalline vitamin B_{12}.

The CHAIRMAN said that the three papers dealt with a subject which had been developed very considerably in the last year or so. If the figures given for assays of commercial extracts really represented their content of vitamin B_{12} then he thought that they gave a very disturbing picture of the state of affairs. The assay process required improvement before it was possible to place reliance on it, but the results given in the papers suggested that there might be some relationship between it and the clinical response. Mr. Shaw used a method of paper strip chromato-