THE GROWTH ACTIVITY FOR *LACTOBACILLUS LACTIS* DORNER OF COMMERCIAL LIVER EXTRACTS

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A Satisfactory method for the standardisation of commercial liver extracts in terms of their anti-anæmic potency would fulfil a long-felt want. Although it has not yet been definitely established that *Lactobacillus lactis* Dorner activity is an exact reflex of anti-anæmic potency, there is a definite correlation between clinical and Dorner effectiveness. The present communication deals with the growth-promoting efficiency for *L. lactis* Dorner of a series of commercial liver extracts of British, American and Continental origin.

Methods of assay used in this investigation are those detailed in the preceding paper[1]. The reference standard used was a solid liver preparation kindly supplied by Dr. Rickes and standardised by him at 0·4 μg of vitamin B_{12} per mg. The results are shown in Table I expressed in μg vitamin B_{12} per g. or ml. It should be emphasised that the values are referred to vitamin B_{12} as isolated by Rickes, and so should be comparable with the values for American liver extracts given by Rickes *et al.*[2]

TABLE I

Number	Manufacturer	Origin	Type	Vitamin B_{12} μg./ml or g.	Remarks
1	A	British	Whole Liver	18·0	Proteolysed
2	A	,,	Oral Liquid	18·0	,,
3	B	,,	Ext. Hepatis Liq. B.P.	14·0	
4	C	,,	,,	13·0	
5	D	,,	,,	9·0	
6	E	,,	Oral Liquid	7·6	Branded plus yeast
7	F	,,	Ext. Hepatis Liq. B.P.	0·5	Labelled Proteolysed
8	G	New Zealand	,,	7·5	
9	A	British	High Potency	10·0	Proteolysed
10	B	,,	,,	13·0	Batch 1
11	B	,,	,,	7·7	Batch 2
12	B	,,	,,	2·5	Batch 3
13	E	,,	,,	0·3	Batch 1
14	E	,,	,,	1·4	Batch 2
15	E	,,	,,	4·5	Batch 3
16	E	,,	,,	5·6	Batch 4
17	E	,,	,,	11·0	Batch 5
18	E	,,	,,	13·0	Batch 6
19	E	,,	,,	16·0	Batch 7
20	F	,,	,,	0·4	Batch 1 labelled Forte
21	F	,,	,,	0·4	Batch 2 labelled Forte
22	H	,,	,,	7·5	
23	I	,,	,,	9·5	Batch 1
24	I	,,	,,	8·0	Batch 2
25	J	U.S.A.	,,	30·0	20 U.S.P. units
26	K	,,	,,	12·0	15 U.S.P. units
27	L	,,	,,	10·0	15 U.S.P. units
28	M	,,	,,	9·0	15 U.S.P. units
29	J	,,	,,	7·4	15 U.S.P. units
30	N	,,	,,	10·0	10 U.S.P. units
31	O	S. America	,,	8·0	
32	P	Continental	,,	30·0	
33	Q	,,	,,	10·6	
34	R	,,	,,	7·4	
35	S	British	Crude Parenteral	3·0	
36	A	,,	,,	3·0	
37	C	,,	,,	2·3	
38	H	,,	,,	3·5	Batch 1
39	H	,,	,,	2·0	Batch 2

A direct comparison has not been made with the anti-pernicious anæmia factor of Lester Smith[3]. Dr. Lester Smith has stated that there might be a slight difference between his preparation and that of Dr. Rickes.

From Table I it will be seen that proteolysed whole liver assays at 18 μg of vitamin B_{12} per g., and that oral liquid liver extracts range from 18 μg. to 7·5 μg. of vitamin B_{12} per ml. It will also be noticed that one oral liver extract, which is stated to have been proteolysed, has little or no activity. Samples of parenteral products prepared by this manufacturer are also exceptionally low in *L. lactis* Dorner factor. From assays performed on comparatively small samples of liver, which had been subject to extensive laboratory treatment with papain, it would seem that proteolysed whole liver contains the entire potency of the liver used. Oral liver extracts in general are but lightly fractionated, and the final potency is probably a function of the extraction efficiency and the degree of accidental natural autolysis.

The vitamin B_{12} content of high potency refined parenteral extracts was found mainly to fall within the range of 7·5 to 16 μg. of vitamin B_{12} per ml. There are two exceptions, one of American and one of Continental manufacture, which assay at 30 μg. of vitamin B_{12} per ml. In a similar manner the crude parenteral liver extracts fall in a group containing 2 to 4·5 μg. of vitamin B_{12} per ml. A disturbing discrepancy was found when different batches of the same brand of high-potency parenteral liver extract were examined. Six batches from manufacturer "E" ranged from 0·3 to 16 μg./ml., three batches from manufacturer "B" varied from 2·5 to 13 μg./ml. These discrepancies were confirmed by repeated assays, taking care to assay the different batches of liver extract on the same day with the same batch of assay medium and against the same standard. In view of the large batch discrepancy shown, a search was made for the possible presence of inhibitory substances in the low-potency extracts. Chromatographic spectra for these extracts were normal with no unexpected increase in potency at any point. If there had been any inhibitory substance present one would not expect this "unknown" to migrate at the same speed as the *L. lactis* Dorner factor. Confirmatory evidence was obtained for the absence of inhibitors by blending low-potency extracts with a solution of crystalline vitamin B_{12}, and assaying the mixture. Values were obtained equal to the sum of the two components assayed separately. A difference would have been expected in the presence of an inhibitor unless the amount of inhibitor was just sufficient to inhibit the activity present in the liver extract alone.

Chromatographic analyses also provide a means of assessing the significance of other growth factor for *L. lactis* Dorner in liver extracts. By upward development there was in some preparations a small amount of activity present in the advance front after 14 days development. This is presumably due to thymidine, but in no case did it amount to more than 5 per cent. of the total Dorner activity. Other batches, although with similar spectra, showed no activity at the advance front. Proteolysed

whole liver, oral liver extracts, highly refined and crude parenteral liver extracts were similar. Downward development in the few samples examined gave entirely comparable spectra except that, since the advance front had proceeded beyond the limits of the strip, no advance front was present. In no case was there any evidence of a significant secondary peak. An illustrative histogram is shown in Figure 1. The absence of subsidiary peaks does not necessarily mean that the four *L. lactis* Dorner active substances described by Lester Smith and Cuthbertson[4] are absent, but provide evidence that they are not present in sufficient quantities to effect the assay.

As a consequence of batch variation considerable doubt is thrown on the utility of assaying one batch of a particular brand as a means of determining the general potency of that particular manufacturer's product. Rickes assayed a number of American liver extracts of an alleged potency of either 10 or 15 U.S.P. units per ml. and found a marked variation between the various brands and also a difference between the various batches of the same brand. The American preparations which have been tested in these laboratories have ranged between 7·5 and

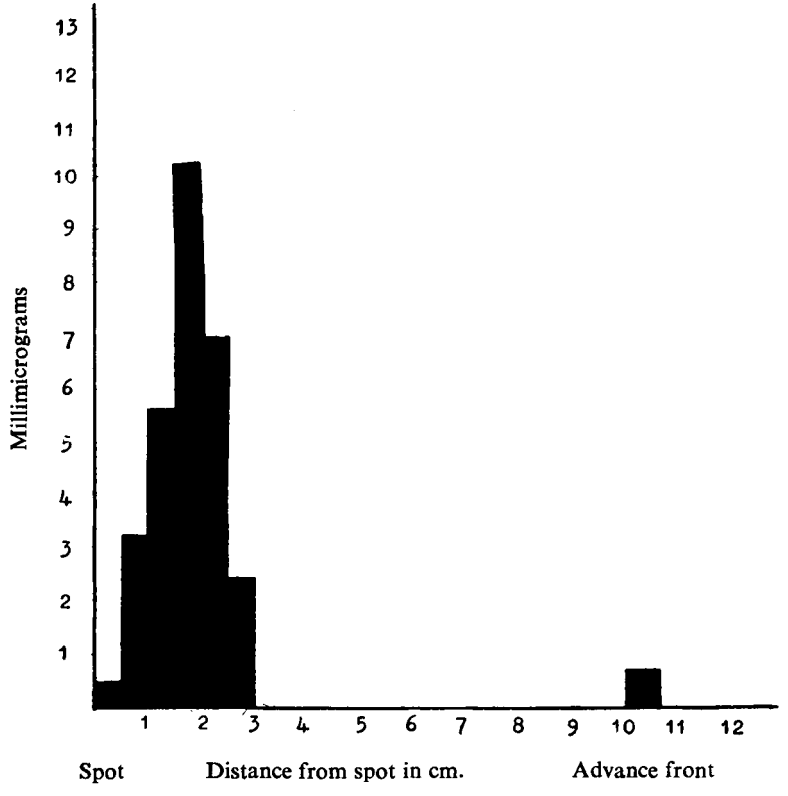

FIG. 1.—Paper chromatograph vitamin B_{12} in liver extract product "B," 0·005 ml. developed for 18 days.

13 μg. of vitamin B_{12} per ml. with the exception of the 30 μg. material already referred to. Manufacturer " F " issues 3 products, a liquid oral liver extract, a parenteral liver extract and a parenteral preparation labelled " Forte." All of these give exceptionally low values, and in this instance one is bound to suspect either the source of liver or else the primary extraction. In the case of manufacturer " E " and " B," both of which are highly reputable British firms, the explanation is not so obvious. Admittedly, one of the low-potency batches is known to be old, but others equally low were bought on the open market for current use. Moreover, we have been unable to find evidence that, in general, finished liver extracts deteriorate in potency on storage even when they have been stored under the adverse conditions which pertain in some of the export markets.

The only reasonable explanation is suggested by work in these laboratories concerned with investigating " process losses " by means of Dorner activity. It is now no secret that in a highly fractionated parenteral extract there may be very appreciable losses during processing, and that the magnitude of these losses may not be constant from sub-batch to sub-batch. Thus, for example, if a certain process is liable to give a 60 per cent. loss, it is not impossible that occasionally this loss is 90 per cent., so that unless a large number of sub-batches are combined in order to " iron out " the variations in process loss, the final product might be only one-third the potency of another.

Summary

1. A number of commercial liver extracts have been assayed for *L. lactis* Dorner activity in terms of vitamin B_{12}. A considerable variation from brand to brand has been found and also a serious variation between different batches of the same brand.

2. Although steps have been taken to eliminate artefacts due to method and the presence of inhibitors, it is not possible at the present time to correlate exactly *L. lactis* Dorner activity with clinical efficacy.

3. It is thus premature to advocate the establishment of a " Dorner " assay unitage applicable to all liver extracts, but it is not too much to expect that, in the future, branded preparations will maintain a reasonably constant " Dorner " activity.

4. The presumptive vitamin B_{12} content of " high potency " liver extracts is of the order of 10 μg./ml. whether of British, American or Continental manufacture.

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References

1. Shaw, *J. Pharm. Pharmacol.*, 1949, **1**,
2. Rickes *et al.*, *Science*, 1948, **107**, 396.
3. Lester Smith and Parker, *Biochem. J.*, 1948, **42**, VIII.
4. Cuthbertson and Lester Smith, *Biochem J.*, 1949, **44**, IV.