THE VITAMIN B_{12} POTENCY OF PHARMACEUTICAL (INCLUDING DIETETIC) PRODUCTS ESTIMATED BY THE OCHROMONAS METHOD

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The vitamin B_{12} potencies of a number of pharmaceutical products were estimated microbiologically using *Ochromonas malhamensis*. The low B_{12} contents found in 9 samples of dried milk, compared with the contents of fresh milk samples, indicated losses during normal drying processes. Rather smaller losses occurred during vacuum drying. In 12 samples of infants' and children's foods the vitamin B_{12} contents ranged from nil to 21 mμg./g., excluding one sample fortified with the vitamin. Storage experiments indicated no significant loss of vitamin B_{12} in dried milk, in foods based on dried milk or in non-allergenic foods based on modified cows' milk or on malt and soya fortified with the vitamin. There was, however, a marked and rapid loss of the vitamin in products which also contained ascorbic acid. This could be prevented by stabilisation of the vitamin with gelatin. Storage experiments on capsules and tablets containing vitamin B_{12} and ascorbic acid did not detect losses of vitamin B_{12} during normal storage in this country. There were marked losses of the vitamin in two brands of capsules, but not in one brand of tablets, during a year's storage in the tropics. These all contained ascorbic acid.

IN a previous communication[1] from these laboratories to the 1956 Conference describing the stability of different B vitamins in pharmaceutical products, attention was drawn to the lack of data on vitamin B_{12}. Apart from spectrophotometric data on injections presented to the 1953 Conference from these laboratories[2], only microbiological methods of varying degrees of specificity were available. However, in 1956 the Analytical Methods Committee of the Society for Analytical Chemistry published[3] a microbiological assay of vitamin B_{12} using the protozoan *Ochromonas malhamensis*, and based on Ford's method[4], with modifications evolved during an investigation by the S.A.C. Vitamin B_{12} Panel, in which our laboratories collaborated. This S.A.C. Ochromonas method has been applied to a variety of foods, feeding stuffs and other biological materials and found to be more specific than the earlier methods using *Escherichia coli* and *Lactobacillus leichmannii* which have previously been used to estimate the vitamin in pharmaceutical products.

METHODS

The S.A.C. Ochromonas method was followed but the growth of the micro-organism was measured, using a Unicam photoelectric spectrophotometer, by the increase in *E*675 mμ, which is at the peak of the chlorophyll absorption curve, as indicated by lower readings at 650 and at 700 mμ. The average coefficient of variation of a single assay was 5.

RESULTS

Dried milk. The vitamin B_{12} content of 6 different commercial samples ranged from 8 to 17 and averaged 13·3 mμg./g. and of 3 different samples of National Dried Milk ranged from 9·3 to 25 and averaged 17·1 mμg./g.

Dietetic specialities based on dried milk. The vitamin B_{12} content of 13 samples ranged from nil to 21 mμg./g. (excluding one apparently fortified containing 46 mμg./g.). In 5 samples also containing eggs the average B_{12} content was 15·3 mμg./g., as compared with an average content of 7·0 mμg./g. in 8 other samples.

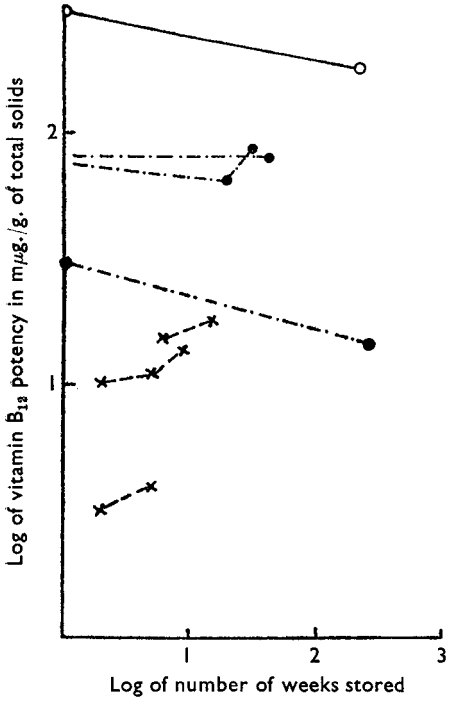

FIG. 1. Stability of vitamin B_{12} under normal storage conditions in dried milk and related foods. Results on dried milk × — — — ×, on non-allergenic foods ● — · — · — ● and on foods deriving vitamin B_{12} from milk and eggs ○——○.

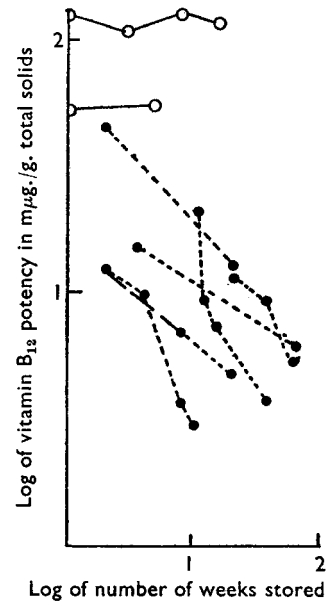

FIG. 2. Stability of vitamin B_{12} under normal storage conditions in foods based on dried milk and containing ascorbic acid. Results before addition of stabilised vitamin B_{12} and stored in closed containers ● — — ●, in open containers ● - - - - ●; after addition of stabilised B_{12} ○——○.

Stability of vitamin B_{12} in dried milk and related foods. Figure 1 shows vitamin B_{12} to be reasonably stable in dried milk during storage periods up to 6 months, in dietetic specialities deriving vitamin B_{12} from milk and eggs during storage for more than 3 years and in non-allergenic foods (*vide infra*) during periods of 1 to 4 years, all at room temperature. On the other hand, as shown in Figure 2, marked rapid losses of both added and natural vitamin B_{12} occurred under normal storage conditions in

dietetic specialities containing ascorbic acid. The rate of loss was slower in an unopened container. Vitamin B_{12} treated with gelatin appears to be stable.

In Figures 1 and 2 the results are plotted on logarithmic scales both for potency and for storage periods in order to enable wide ranges to be included. As the precise ages of some of the samples were not known the positions on the time scales of some of the points are approximate only. This does not affect the marked difference between the relatively stable sources of vitamin B_{12} as shown in Figure 1 and the very unstable sources of the vitamin as shown in Figure 2.

Non-allergenic foods. These are given to infants to combat allergies to cow's milk. If the latter is modified by removal of lactose, for treatment of galactosaemia, or of some of the albumen, for treatment of asthma or eczema, considerable losses of different B vitamins may occur. The vitamin B_{12} content may fall to less than 12 or even less than 10 mμg./g. total solids. In 4 commercial samples of non-allergenic foods based on malt and soya which have proved rather more successful in the treatment of these allergies, vitamin B_{12} added at levels of 50 to 85 mμg./g. was found to be reasonably stable during prolonged storage under normal conditions.

Other pharmaceutical products. Vitamin B_{12} occurs in other pharmaceutical products such as liver powder and in various multivitamin preparations including capsules and tablets in which it is supplied either as liver extracts or liver powder or as the pure vitamin. Our tests on a few of these products indicate that the stability of the vitamin is usually satisfactory during normal shelf life in this country. During a year's storage in the tropics losses of 80–100 per cent of the claimed vitamin B_{12} content occurred in 2 brands of multivitamin capsules also containing ascorbic acid, although in tablets containing similar amounts of vitamins B_{12} and C no significant losses were detected.

Discussion

The average vitamin B_{12} content of the samples of dried milk we have examined was only 15 mμg./g. total solids. A recent survey[5] indicated average B_{12} contents of fresh milk ranging from 3 to 6·6 mμg./ml., equivalent to 24 to 50 mμg./g. total solids. Since the vitamin B_{12} in dried milk was found to be reasonably stable, the low B_{12} content of our dried milk samples did not seem likely to be due to losses during storage, but more probably to losses during drying. Some experiments carried out on vacuum drying of milk for 3–4 hours at 40–45° indicated losses of about 10 per cent, and we therefore think that greater losses might occur during drying at atmospheric pressure.

Milk does not normally contain significant amounts of ascorbic acid. If this be added in sufficient quantities to meet human requirements, both natural and added vitamin B_{12} may be rendered unstable and disappear rapidly during storage under normal conditions. Since ascorbic acid reacts at a suitable pH (4–6) with hydroxocobalamin but not with cyanocobalamin[2], the destructive action of the ascorbic acid might be prevented or at least retarded by ensuring that the vitamin B_{12} was all present as

cyanocobalamin. This could be done by combining the cyanocobalamin with a stabilising agent such as protein. We have found gelatin satisfactory for this purpose. It is now being tried for the stabilisation of vitamin B_{12} in multivitamin capsules and tablets containing ascorbic acid. Whilst we encountered losses of vitamin B_{12} in the capsules, though not in the tablets, during storage in the tropics, Campbell and McLeod[6] encountered losses in the tablets but not in the capsules, during storage in Canada.

We are indebted to Mrs. Susan Davis and Miss Jacqueline Lloyd for assistance, also to Messrs. R. P. Scherer Ltd. for a supply of stabilised vitamin B_{12}.

Footnote

After this paper had been prepared for the press Dr. J. E. Ford kindly showed us the typescript of a paper from the National Institute for Research in Dairying, and the Dairy Department, University of Reading, by H. Chapman, J. E. Ford, S. K. Kon, S. Y. Thompson and S. J. Rowland, also E. L. Crossley and J. Rothwell, describing results (which are being published in the *Journal for Dairy Research*) in substantial agreement with our findings on vitamin B_{12} in milk. They indicate vitamin B_{12} contents of 13 to 28 mμg./g. in National Dried Milk and marked losses of the vitamin in the manufacture of condensed and evaporated milk.

References

1. Wokes and Norris, *J. Pharm. Pharmacol.*, 1956, **8**, 895.
2. Baxter, Horsford, Wokes, Norris and Fernandes, *ibid.*, 1953, **10**, 723.
3. *Analyst*, 1956, **81**, 132.
4. Ford, *Brit. J. Nutr.*, 1953, **7**, 299.
5. Gregory, *Dairy Sci. Abs.*, 1955, **17**, 174.
6. Campbell and McLeod, *J. Amer. Pharm. Ass., Sci. Ed.*, 1955, **44**, 263.

DISCUSSION

The paper was presented by Dr. F. Wokes.

Mr. N. J. Van Abbé (Loughborough). Was roller-dried or spray-dried milk used?

Mr. K. A. Lees (London). Had the results been checked by some comparative technique?

Dr. G. E. Foster (Dartford). What explanation could be offered for the apparent instability of vitamin B_{12} in the presence of ascorbic acid? Why did capsules show loss of vitamin B_{12} whereas tablets did not?

Miss A. E. Robinson (London). It should be possible by differential absorption techniques to determine the relative proportions of scatter and absorption.

Mr. H. J. Bragg (Folkestone). How many determinations were made over the period, and were the capsules and tablets stored in the original containers and accelerated tests carried out?

MR. G. R. WILKINSON (London). The logarithmic scale (Figure 1) compressed what were wide differences. Had the authors any explanation why some samples appeared to increase in vitamin B_{12} content?

MR. K. A. LEES (London). The authors referred to a discrepancy between their results and those of Canadian workers, had the Canadian workers used the same type of capsules?

MR. D. E. WILKS (Bedford). Was the fresh milk, milk straight from the cow or pasteurised; were there any details available about the vitamin B_{12} content of pasteurised milk?

DR. F. WOKES replied. He was not aware how the milk had been dried. They were commercial samples. The limits of reliability were indicated and all the points obtained were in the curves. A Spekker absorptiometer had also been used, and good agreement was found between the two methods. The reaction between ascorbic acid and vitamin B_{12} depended on cyanocobalamin being first converted to hydroxycobalamin. The composition of the capsules and tablets varied and he did not know why deterioration occurred only in the capsules. Opacity methods were accepted methods for measuring growth; if known amounts were taken, and calibrations made, the response was proportional to the log of the concentration of the vitamin. There was a linear response curve. Storage experiments were made on the capsules in the original bottles and packets. An accelerated storage experiment had been made, and it had been found that at above 37° the vitamin became much less stable; also if too high a temperature were used the physical nature of the gelatin altered. If the moisture content were low the gelatin became brittle. In tropical conditions the reverse might happen, and the shell of the capsule might become more permeable. The logarithmic scale was chosen for the graphs in order to include data over a wide range in one diagram. The variations were puzzling. In two or three points in Figure 1 there was an apparent increase. In some cases the rise was rather large for experimental error. Some samples were available five or six years ago, and they were made when crystalline vitamin B_{12} first became available. Of the capsules examined, some were oil-filled and others dry-filled. Tablets which were perfectly dry had also been examined. The samples were of fresh milk. Pasteurised milk contained little vitamin B_{12}.