STABILITY OF B VITAMINS IN PHARMACEUTICAL PRODUCTS

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The standardisation of the vitamin B contents of pharmaceutical (including dietetic) products has been greatly facilitated by the development of physico-chemical and microbiological assay methods. These, as shown by comparison with the results of biological assays, can be relied upon to ascertain that the vitamin contents claimed are, in fact, present when the products leave the manufacturer. Much further information is, however, still needed by the pharmaceutical and medical profession about possible loss of vitamin potency during different storage conditions in the pharmacy, hospital or home. A certain amount of such information has already been given in publications dealing with vitamins A[1,2] and C[3–5]. In a recent paper[6] dealing with the content of A, B and C vitamins in samples of multivitamin products purchased in Canada, references are given to some results on the stability of B vitamins in pharmaceutical products obtained by several American and Canadian workers. Apart from the work of Parkington and Waterhouse[7] on the stability of different aneurine salts, very little appears to have been published about the stability of the B vitamins in pharmaceutical products under the storage conditions in general practice in this country.

Methods

Products Examined

Our findings are based on over 10 years' experience in the development of physico-chemical and microbiological assays and their application to a wide range of pharmaceutical products. To simplify the presentation of our data, we give here only our results on the following four classes of products.

(*a*) Vacuum dried dietetic specialities claiming vitamins in which the significant sources of B vitamins are usually malt and milk. One food concentrate which we have studied extensively also contains eggs. Some fortification with synthetic vitamins may be employed to allow for variations in the vitamin B contents of the raw materials.

(*b*) Bakery products in which B vitamins are obtained from cereals, with more extensive fortification than in (*a*).

(*c*) Vitamin concentrates prepared *in vacuo* in which the B vitamins are obtained from malt and in one product also from yeast. Fortification with B vitamins is employed.

(*d*) Multivitamin capsules* in which the B vitamins are supplied mostly in synthetic form in different media.

* After this paper went to press, H. E. F. Notton published (*Pharm. J.*, 1956, **177**, 69) further data on the stability of vitamins in capsules.

Our results were obtained on less than 20 different products out of more than a hundred on the market, and therefore cannot cover all the variations which occur in practice, though they may perhaps provide useful representative data.

Storage Conditions

The various products under investigation were stored, in the containers in which they are normally sold, in incubators at 27°, 37° or 43° C., in a refrigerator at about 3 to 4° C., and at room temperature. The relative humidity was below 70 per cent. except in certain experiments in which it was raised to 90 to 100 per cent. by means of suitable salt solutions. To test the effects of the fluctuating temperatures and humidities in the tropics, a number of field trials have been arranged in which samples are sent out to different tropical countries for periods ranging from 6 to 18 months for storage under the prevailing conditions, and then returned for examination.

Assay Methods

Vitamin B_1. Fluorimetric assays were by the S.P.A. method[8] with certain slight subsequent modifications. Microbiological assays were by the method of Fitzgerald and Hughes[9], with modifications proposed in the Report of the Thiamine (Microbiological) Panel of the S.P.A.[10], using *Lactobacillus fermenti* 36.

Riboflavine. Fluorimetric assays were by the method of Klatzkin, Norris and Wokes[11], and microbiological assays by the method proposed in the Report of the Vitamins Estimation (Microbiological) Panel of the S.P.A.[12], using *L. helveticus.*

Nicotinic Acid. Chemical assays were by the method of Klatzkin, Norris and Wokes[13]. Microbiological assays were by the method proposed in the Report above (for Riboflavine), using *L. arabinosus* 17/5.

Vitamin B_6 was assayed by the microbiological method using *Neurospora*[14], *Pantothenic acid* was assayed by the microbiological method using *Lactobacillus arabinosus*[15], Biotin was assayed by the microbiological method using *Lactobacillus arabinosus*[16], *Folic acid* was assayed by the microbiological method using *Streptococcus fæcalis*[17], and *Vitamin B_{12}* by the microbiological method using *Ochromonas malhamensis*[18].

At least two assays were carried out on each sample. If the results were not in sufficiently good agreement, more assays were made.

Results

Vitamin B_1

Dietetic specialities. Figure 1 shows that in the food concentrate stored in this country in tins with air-tight metal closures, there was no appreciable loss of vitamin B_1 even after more than 7 years' storage at room temperature, and the content always remained well above the claim. In other products without air-tight metal seals the rate of loss ranged

from 6 to 20 per cent. in 3 to 8 years at room temperature. At 37° C. the food concentrate in the metal sealed tins showed a very slow rate of loss, barely reaching 15 per cent. in 7 to 8 years. In the other products without metal-sealing, the rate of loss was increased, and after a year or two the vitamin content had fallen below the claim.

Bakery Products. In bakery products, vitamin B_1 is generally stable, both at room temperature and at 37° C., no appreciable loss being observed during over five years storage under reasonably dry conditions (relative humidity below 70 per cent.), whilst the content remained well above the claim (Fig. 2). The effect of baking on the vitamin does not seem to render it any less stable[19].

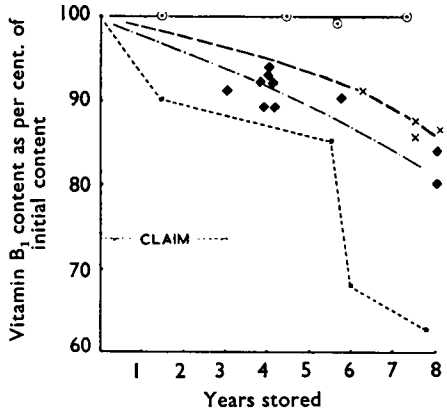

FIG. 1. Rate of loss of vitamin B_1 in vacuum-dried foods:

Stored at room temperature in hermetically-sealed tins .. .. ⊙——⊙
Stored at room temperature in containers not completely air-tight .. ◆-·-◆
Stored at 37° C. in containers not completely air-tight .. .. *----*
Stored at 37° C. in hermetically sealed tins .. .. ×---×

The initial vitamin B_1 content of the food concentrate averaged 14·2 μg./g. (as compared with a claim of 10·6 μg./g.) and of the other products ranged from 3 to 12 μg./g., with smaller "overages" than on the food concentrate.

Vitamin concentrates. The higher moisture content of vitamin concentrates seems to render the vitamin B_1 rather less stable (Fig. 3). Although the initial vitamin B_1 content may be 20 per cent. above the claim (this "overage" being provided to allow for the possible greater loss during storage), it is advisable to store the product in a cool place in order to ensure a shelf life of about 2 years. At 4 to 5° C., the rate of loss may be only about 5 per cent. in 2 years, provided that the product is stored in the original air-tight and completely filled glass containers. When these are opened to remove some of the contents, the rate of loss in the remainder is increased, but the vitamin B_1 concentration should not be significantly affected before the whole is consumed. In the original unopened jars stored at room temperature, the vitamin B_1 content of products with an overage of 20 per cent. should not fall below the claim until $2\frac{1}{2}$ to $3\frac{1}{2}$ years have elapsed.

Multivitamin capsules. The administration of vitamins in capsules raises a number of problems concerned with external factors such as storage temperature and exposure to light, moisture, etc., and also with internal factors such as the presence of minerals or other interfering substances in the capsules. The stability and assimilation of the vitamins

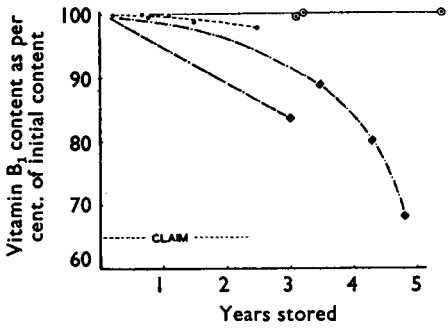

FIG. 2. Rate of loss of vitamin B_1 in bakery products:

Stored at room temperature in air-tight containers .. .. .. ⊙——⊙
Stored at room temperature in containers not completely air-tight .. ◆-·-◆
Stored at 37° C. in air-tight containers *- - - -*

The initial vitamin B_1 content averaged 28 μg./g. (as compared with a claim of 17·6 μg./g.) in the main product investigated. In the other products, it ranged from 16 to 28 μg./g., with smaller "overages .

ing, most of the above disturbing factors have been eliminated. The possible deleterious effect of light on the contents is prevented by a pigment in the capsule shell. No minerals are present to interfere with stability or assimilation, and the B vitamins are rendered more stable by their incorporation in a special yeast medium. Our findings, will, therefore, not necessarily apply strictly to capsules in which such precautions have not been taken.

In this country, the capsules when stored at room temperature under the usual conditions retain their vitamin B_1 content remarkably well, no loss having been detected after several years (Fig. 4). In tropical climates, may be affected by minerals present. Vitamin C, for example, is less stable at a pH value above 6. The capsule shells may, under certain conditions, absorb some of the vitamins which, however, may still be available to the patient. But some vitamins may be lost by leakage if the shell becomes softened by atmospheric moisture as in humid tropical climates. On the other hand, intense dry tropical heat may make the shells brittle which could lead to leaks if the capsules were shaken too vigorously in their containers.

In the particular multivitamin capsules which we have been mainly investigat-

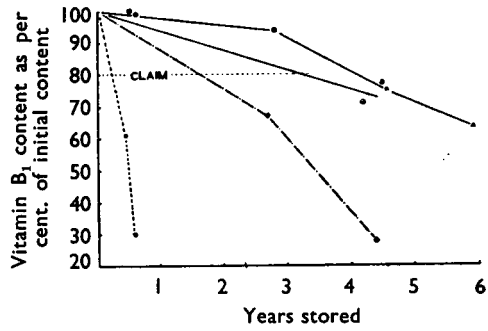

FIG. 3. Rate of loss of vitamin B_1 in vitamin concentrates:

Stored at 3–4° C. in completely filled jars .. .. ●——●
Stored at 3–4° C. in partly filled jars ▲——▲
Stored at room temperature in completely filled jars .. .. .. ⊙——⊙
Stored at room temperature in partly filled jars .. .. ◆-·-◆
Stored at 37° in partly filled jars .. *- - - -*

The initial vitamin B_1 content averaged 15·4 μg./g. (as compared with a claim of 12·3 μg./g.) in the main product investigated. In the other products, it ranged from 7 to 15 μg./g., with smaller "overages".

the rate of loss varies widely according to the climatic conditions. If the capsules are kept in dry, sealed containers, temperatures up to 43° C. have had only slight effect on the vitamin B_1 content, so that the claim may still be met even after 2 years. If the capsules are not stored in absolutely air-tight containers, the rate of loss increases with the relative humidity, and may reach about 20 per cent. per annum. Significant losses of vitamin B_1 during storage in the tropics can be avoided only by stringent precautions.

When prolonged storage under unsuitable conditions has led to an appreciable loss of vitamin B_1 in foods and pharmaceutical products, substances may be formed which exert quenching effects and thus lower the per cent. recovery in fluorimetric assays. If the fluorimetric results are adjusted by means of this recovery (i.e., by multiplying by 100 and dividing by the per cent. recovery), the fully adjusted results are too high as can be shown by comparison with microbiological results (Table I), so that storage losses may be masked and escape attention when the usual fluorimetric procedures are adopted. This difficulty can be overcome by using a factor which has to be determined separately for each type of food by a method described elsewhere[20]. For food concentrates of the type we have been investigating, the factor has been found to be about 0·3. If the factor of 0·3 is multiplied by the increase in the result which would be obtained by making full adjustment for the per cent. recovery, a quantity is obtained which when added to the unadjusted result will give a value close to the true value.

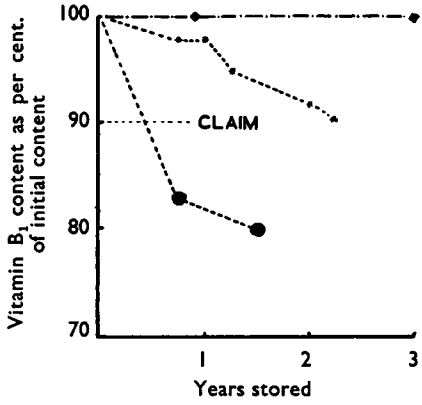

FIG. 4. Rate of loss of vitamin B_1 in multivitamin capsules:

Stored at room temperature in tins .. ◆–·–◆
Stored at 37° C. in screw-capped, sealed bottles .. .. .. *– – – –*
Stored at 37° C. in screw-capped sealed bottles at 90 to 100 per cent. relative humidity .. .. .. ●– – –●

The initial vitamin B_1 content averaged 2·75 mg. per capsule (as compared with a claim of 2·5 mg.) in the main product investigated. In the other products it ranged from 0·3 to 1 mg. per capsule, with similar "overages".

Riboflavine

Dietetic specialities. In samples of the food concentrate stored in the usual metal-sealed tins under normal conditions, the losses of riboflavine after 2 to 3 years have been found to lie between 5 and 10 per cent. In Figure 5, the riboflavine contents are given as per cent. of the initial content, and not in relation to any claimed content, since such claims are not yet being made for the food concentrate in this country. When the tins

have been opened to remove some of the contents, the rate of loss may be increased, so that after little more than a year's storage, the loss has become significant. Storage at tropical temperatures (37–43° C.) does not greatly increase the rate of loss in unopened tins. The metal-sealing, therefore, appears to be sufficient to prevent significant loss of riboflavine even during prolonged storage under tropical conditions, and the main precaution to be adopted is to avoid exposing the contents to air for too long a period after the tins have been opened. If such exposure does occur during storage in the home, it may be detected by the caking of the contents.

TABLE I

COMPARISON OF FLUORIMETRIC AND MICROBIOLOGICAL ASSAYS OF VITAMIN B_1 IN A FOOD CONCENTRATE (ALL RESULTS AS μG./G.)

Age of sample years	Fluorimetric results		Microbiological results
	Adjusted for full recovery	Adjusted using factor	
4	14·0	14·0	14·0
2	13·9	13·4	13·7
3	14·2	14·0	14·6
2	16·6	14·0	13·7
4	18·4	14·4	13·7
Means	15·4	14·0	13·9

Bakery products. Under normal storage conditions, the rate of loss of riboflavine was found to be only 3 to 6 per cent. per annum, so that even after 3 years the content exceeded the claim (Fig. 6). Where there was exposure to a moist atmosphere, the riboflavine became less stable. In other storage experiments at 27 and 37° C., the rate of loss under satisfactory conditions was not much higher than at room temperature provided that there was no undue exposure to a moist atmosphere. In order to simplify the presentation of the data, these results have been omitted from Figure 6.

Vitamin concentrates. In the product we have mainly investigated, the stability of riboflavine was better than that of vitamin B_1 during storage in the original air-tight completely filled jars. Thus at 37° C., there might be only about 10 per cent. loss in 3 years, and at room temperature practically no loss after nearly 5 years' storage (Fig. 7). When the jars were opened and some of the contents removed, the riboflavine content still remained above the label claim throughout the time taken to consume the whole of the contents.

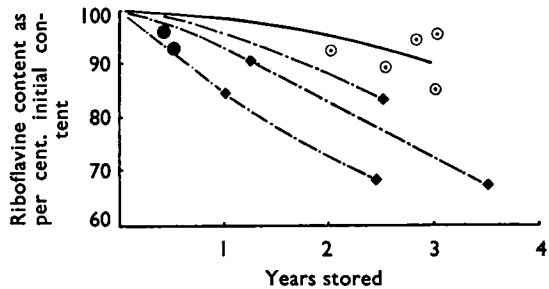

FIG. 5. Rate of loss of riboflavine in vacuum-dried foods:

Stored at room temperature in hermetically-sealed tins .. .. ⊙——⊙

Stored at room temperature in containers not completely air-tight .. .. .. ◆-·-◆

Stored at 37 to 43° C. in hermetically-sealed tins .. .. ●

The initial riboflavine content ranged from 3 to 20 μg./g. in the different products examined, with "overages" of 5 to 15 per cent.

As with vitamin B_1, prolonged storage under unsuitable conditions may lead to the development of substances which exert quenching effects in fluorimetric assays of riboflavine, thus lowering the recovery of added riboflavine. If this percentage recovery is used to adjust the fluorimetric results, the fully adjusted results may be too high so that the loss of vitamin during storage is obscured. When this difficulty is overcome by application of a factor, which has to be specially determined for each type of food, the partially adjusted results then become nearer to the true values, indicated by microbiological assays.

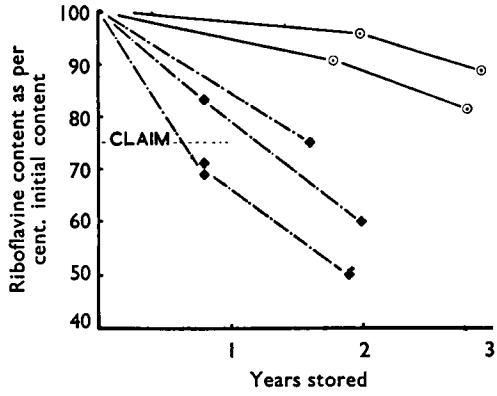

FIG. 6. Rate of loss of riboflavine in bakery products:
Stored at room temperature in air-tight containers .. .. ⊙——⊙
Stored at room temperature in containers not completely air-tight .. .. .. ◆- - -◆

The initial riboflavine content ranged from 5 to 30 μg./g. in the different products examined, with "overages" of 5 to 20 per cent.

Multivitamin capsules. Our findings emphasise the importance of avoiding undue exposure to atmospheric factors during the manufacture and storage of pharmaceutical products containing riboflavine. The problem is simplified when we turn to multivitamin capsules, in which the vitamins are protected from the action of air. The riboflavine in the multi-vitamin capsules we have been investigating did not undergo any significant loss during more than a year's storage under normal conditions at 37° C.

Nicotinic acid

This vitamin, in the form either of acid or of amide, has been found to be more stable than vitamin B_1 or than riboflavine. No significant losses have been detected either by chemical or by microbiological assays in any of the four classes of products we have been studying, when stored under normal conditions in this country for several years, even in opened containers. Thus, in one sample of the food concentrate stored at room temperature in opened tins for 4 years, a loss of about 7 per cent. was indicated by our assays, but this did not exceed the combined experimental errors of the assays. In another sample also stored in an opened tin at room temperature for 6 years, an increase of the same order was encountered, and it therefore seems safe to assume that the claim for nicotinic acid in this product will be met even when the storage period greatly exceeds the normal shelf life. Our experimental findings indicate that this applies also to bakery products

and vitamin concentrates. No losses of nicotinic acid have been detected in multivitamin capsules during several years' storage at room temperature. When the capsules were stored under tropical conditions at 37 to 43° C., losses of 6 to 16 per cent. were found in 1 to 3½ years' storage. However, the capsules had, under these conditions, ruptured the shells and released some of the contents, hence were obviously unsuitable for administration. This can be prevented by storage under suitable conditions.

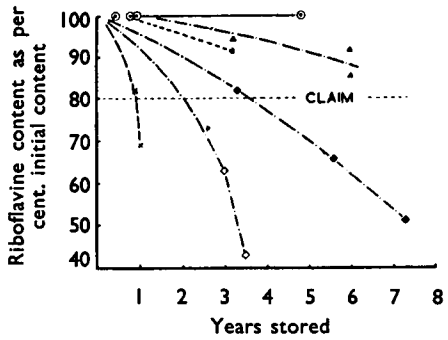

FIG. 7. Rate of loss of riboflavine in vitamin concentrates:

Stored at 3 to 4° C. in partly filled jars ▲–·–▲
Stored at room temperature in partly filled jars .. .. ◆–·–◆
Stored at room temperature in completely filled jars .. .. .. ⊙——⊙
Stored at 37° C. in completely filled jars .. .. *– – – –*
Stored at 37° C. in partly filled jars .. ×– – –×

The initial riboflavine content ranged from 5 to 12 μg./g. in the different products examined, with "overages" of 5 to 15 per cent.

Other B vitamins

The above mentioned vitamins (B_1, riboflavine and nicotinic acid) have for over 10 years been recognised by the Ministry of Food as being essential to health and liable to be deficient in human diets. Hence, they have been scheduled in the Labelling of Food Order (1946). During the last few years, claims have been made in increasing numbers for the presence in pharmaceutical products of other B vitamins which are not yet recognised by the Ministry of Food, although the Ministry of Health does not always frown on their being prescribed. Whilst our experience with these has been less extensive, their properties and stability are also important.

Vitamin B_6

Suggestions have recently been made[21] that deficiencies of this vitamin in low extraction flours may accentuate deficiencies of essential fatty acids, and its occurrence and stability in pharmaceutical products may, therefore, receive more attention in the future. In neutral or alkaline solution, vitamin B_6 is sensitive to ultra-violet radiation and suitable protection may, therefore, be needed for certain liquid or semi-liquid preparations, such as might be administered in multivitamin capsules. However, no losses of vitamin B_6 potency have been detected in the particular products we have been studying during storage for over a year at normal temperatures.

Pantothenic acid

Unequivocal pantothenic acid deficiency has not yet been reported in man, though it probably occurs as a complication in beri-beri and pellagra.

Results of microbiological assays show no significant losses of pantothenic acid during 18 to 20 months storage at room temperature in the products which we have studied.

Biotin

Biotin deficiency may occur in man. This B vitamin appears to be stable to heat, acids and alkalis, and no losses have been detected (microbiological assays) during 18 months storage at room temperature.

Folic Acid

Folic acid deficiency has been found in man, but on the other hand, administration of large doses of folic acid in vitamin B_{12} deficiency may precipitate neurological manifestations, hence the intake of both of these vitamins should be considered together. No losses of folic acid potency have been detected during 18 months storage at room temperature in those of the products we have been studying.

Vitamin B_{12}

The stability of this vitamin in the injections used for treatment of pernicious anæmia has been considered in a previous communication[22] from our laboratories giving results obtained both by spectrophotometric and by microbiological assays. For preparations given by mouth, the evidence rests entirely on microbiological assays, and is more scanty. However, in the products we have so far investigated, the vitamin B_{12} has been found to be stable for 18 months or more, provided that suitable precautions are taken. The occurrence and stability of vitamin B_{12} in dietetic products may receive more attention in the future, since human dietary deficiency of vitamin B_{12} has recently been reported[23] amongst persons (termed "vegans") living in this country on diets containing no animal food, not even milk or eggs.

Discussion

Our findings on the four different types of products indicate that the main precautions to be taken during their storage involve avoidance of exposure to atmospheric conditions, especially in hot humid climates. In certain circumstances (e.g., when riboflavine or other photo-labile vitamin is present) protection against the action of light is also desirable. Maximum stability is, of course, best achieved by storing these products in air-tight containers in a cool, dry, dark place. If the containers are opened and some of the contents removed, losses of B vitamins may set in after sufficient atmospheric moisture has been absorbed. The amount of air space above the contents can then become important, since it may diminish the stability of the riboflavine.

Campbell and McLeod[6] encountered serious losses of pantothenic acid in 2 out of 3 brands of multivitamin capsules stored for different periods up to 19 months under conditions not precisely stated. In the third brand they found very little loss, which is in agreement with our findings.

Our experiments on dry products detected no significant losses of pantothenic acid during 18 to 20 months storage at room temperature.

This is in agreement with the findings of Campbell and McLeod on one make of tablet. Their results on the other three makes of tablets which they were studying would have been interesting. They also agree with our findings that other B vitamins are reasonably stable in capsules, so that "overages" of 10 to 20 per cent. should be sufficient to ensure that the claims were met during a normal shelf life. Our experience on the stability of B vitamins in liquid multivitamin products has been rather limited. However, we agree with Campbell and McLeod in finding vitamin B_1 in these to be much less stable than in dry products.

This discussion would not be complete without some reference to the "overages" or excess vitamin contents initially provided by manufacturers to allow for losses during storage. Campbell and McLeod do not mention these in their paper, although their data show that appreciable "overages" must have been provided in most of the products they investigated. Examples of "overages" in actual use in this country are given in the present paper. Without a knowledge of the "overages" of the different vitamins initially supplied in any product, it is obviously not possible to make precise calculations about losses during storage, or to make any strict comparison of the relative stabilities of the vitamins in different products.

Campbell and McLeod have suggested that the manufacturer should be responsible for the maintenance of the potency of vitamin products over their normal shelf life. In our opinion, this responsibility can best be met by supplying the product in satisfactory containers, with advice to the pharmacist on the most suitable storage conditions, and by providing a sufficient "overage" of the different vitamins to ensure that the claims will still be met after a reasonable period of storage.

SUMMARY

1. Storage experiments extending over 3 to 8 years have been carried out on representative samples of vacuum dried, dietetic specialities, bakery products, vitamin concentrates with a basis of malt extract and multivitamin capsules in which B vitamins have been claimed.

2. Changes in the contents of these vitamins have been followed by physico-chemical and microbiological assays.

3. The stability of the vitamins has been found to be affected by exposure to air as well as by temperature and relative humidity.

4. In vitamin concentrates with a malt extract basis, storage in a cool dry place is advocated to maintain the vitamin content during a reasonable shelf life. (1–2 years, depending on the "overages" provided.)

5. In multivitamin capsules, the stability of the vitamins may be affected by light as well as by exposure to high temperatures and relative humidity, and by interaction with other constituents. When precautions are taken to avoid these disturbing factors, the B vitamins in the capsules should not undergo significant loss during a reasonable shelf life.

We are indebted to a number of colleagues, including Miss Elaine Morphet, Miss Maureen Metcalfe and Mrs. Sheila Cowen, for assistance

in carrying out the considerable number of assays required for this investigation.

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DISCUSSION

The paper was presented by DR. F. WOKES.

DR. G. E. FOSTER (Dartford) referring to the assay of vitamin B_1, said it was well known that under some conditions, particularly in multivitamin capsules, there could be a decrease in the vitamin B_1 content on storage. It was possible that the decomposition products in the preparation under examination might produce some impurity which would quench the fluorescence of the thiochrome solution.. It would be rather serious if the method used was unreliable. Could the authors give any information as to whether quenching interfered with the estimation?

DR. D. C. GARRATT (Nottingham) suggested that the paper might preferably be entitled "Stability of B vitamins in *Certain* Pharmaceutical Products". The preparations were obviously specialised, and he would hesitate to use the information in the paper as a basis for generalising on the stability of the B vitamins in pharmaceutical preparations. It was fairly well known that in spite of reasonable precautions in storage, these substances did not remain stable over the length of time set by the authors, and it would be interesting to hear of further experience with normal vitamin products.

MR. E. H. B. SELLWOOD (London) asked whether the authors had any information on the relative stability of aneurine chloride or nitrate, and riboflavine free or as phosphate.

MISS J. ASHWIN (Dorking) said that in the case of pantothenic acid it would be interesting to know whether any assays had been made after storage at higher temperatures, particularly of vitamin concentrates. She asked whether the authors could comment on the stability of vitamin B_{12} in liquid preparations containing vitamins B_1 and C.

DR. F. WOKES quoted extracts from a letter received from Dr. Campbell in Canada, in which he confirmed the findings of the authors and suggested the use of expiration data on container labels. Such data must be based on stability studies of each product.

DR. F. WOKES, in reply, said that the point raised by Dr. Foster had been dealt with to some extent in Table I, where it was shown that samples of different ages gave different recoveries, the lowest being in the older samples. In a previous paper a method had been described of allowing for recovery by using a factor which had to be determined separately for each type of product. In Table I of the paper under discussion it was shown that if such a factor were used, the average was close to the biological assays. The problem could alternatively be overcome by diluting the solutions, thereby diluting out the fluorescence. It had been shown that by sufficient dilution it was possible to raise the percentage recovery to nearly 100 per cent. and thus no correction was necessary. He accepted the criticism concerning the limitation of the work to *certain* pharmaceutical products, but pointed out that the paper stated clearly that only a limited number of products were covered which included not only specialities but some which were in the B.P. The authors had no data about the relative stability of aneurine in the form of different salts except for the hydrochloride. A good deal of the aneurine came from natural sources and would be in combined organic form. He had no information on the stability of pantothenic acid. The preparations tested for vitamin B_{12} were solids and he had no data on the stability of this vitamin in liquid preparations containing vitamins B_1 and C.