CCXXXII. THE WATER-SOLUBLE B-VITAMINS IN YEAST, FLOUR AND BREAD

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BREAD made from wheaten flour and yeast is one of the most widely distributed forms of food. With the possible exception of rice and maize it enters into the diet of more people than does any other single foodstuff, and in many cases, owing to its cheapness and high calorie value, it is the chief constituent of the diet. This being so, it is natural that the question of its nutritive value should be a matter of importance.

Whole cereals contain fairly considerable amounts of the B-vitamins, but as the germ and pericarp are relatively richer than the endosperm, and these are removed in milling, a great part of the vitamin value of cereals is lost. A high proportion of cereal products, poor in B-vitamins, may normally be supplemented by other foodstuffs in a varied diet, but in times of economic stringency, when satisfaction of the calorie requirements is the first consideration, these supplementary foods will be omitted, and a deficiency of B-vitamins may occur.

Two conditions which have been shown to develop on cereal diets deficient in the B-vitamins are beriberi and pellagra, the first arising when there is a low intake of vitamin B₁ and the second when there is a low intake of some part of the vitamin B₂ complex. Although beriberi is in general associated with the eating of highly milled rice, which has been shown to contain little or no vitamin B₁, and pellagra is associated with the consumption of maize, yet there are well-established instances of both these diseases occurring among those whose staple diet was white wheaten bread [see Siler *et al.* 1913; Aykroyd, 1930]. This would seem to imply that white bread is not a good source of the B-vitamins, though the fact that these instances are relatively few would indicate that it may be a better source than some other cereal preparations.

Observations such as these do not provide material for accurate deductions as to the adequacy of wheat products as a source of the B-vitamins for human beings, and it is obvious that the matter will not be determined until both the exact vitamin contents of breads and the human needs for the vitamins are known.

The following work is an attempt to add a few facts to the above data. It cannot be regarded as complete or final, indeed during the course of the work it became apparent that a further differentiation of a supposedly simple member of the B-vitamin series into two factors had rendered at least a part of the work of doubtful use, but it is hoped that it may be a further step in the elucidation of the nutritive value of bread, a problem of major importance to a nation of bread eaters.

MATERIALS

Yeasts

These tests for the B-vitamins in yeasts were made in order to discover if there were considerable variations in different samples of yeasts and also to see whether yeast would be a suitable material to use as a standard for the vitamin

B₂ complex and its constituents, flavin and factors other than flavin, since there are as yet no international standards for these vitamins such as exists for vitamin B₁.

The following yeasts were tested:

Brewer's yeast (dry weight 20 %) from Messrs Watney, Combe, Reid & Co.

Baker's yeast I (dry weight 27 %) Distillers' Co. ordinary.

Baker's yeast II (dry weight 32 %) Distillers' Co. reinforced with vitamin B₁.

Baker's yeast III (dry weight 28 %) Distillers' Co. green label.

The baker's yeast III contained 28.4 % solids (dried at 37°) and 25.82 % (dried at 100°). This and the other yeasts were fed after drying at 37°.

Flours

The flours tested were milled from two samples of blended wheats, which were obtained with the co-operation of Dr Kent-Jones of Dover. Their composition was as follows:

<i>Sample A.</i>	25 % No. 2 Manitoba	<i>Sample B.</i>	12 % Persian
	25 % No. 5 Manitoba		20 % No. 2 Manitoba
	50 % Yeoman		12 % No. 3 Manitoba
			37 % Plate
			19 % Bohemian

From *Sample A* were milled:

- (1) a wholemeal flour consisting of the whole grain;
- (2) a straight run flour consisting of 70–72 % of the whole grain. This flour could be taken as representative of that used in this country for making white bread. The sample was divided into two parts, one of which was untreated and the other was bleached with nitrogen peroxide.

From *Sample B* were milled:

- (1) a straight run flour consisting of 70–72 % of the whole grain;
- (2) a patent flour; this was what is commercially known as a "top 60", it consisted of the top 60 % of a straight run flour and so represented 42 % of the whole grain.

These samples were not bleached.

Analyses of these flours were kindly carried out for us by Dr Kent-Jones. The water, ash and nitrogen values are shown in Table I.

Table I. *Composition of the flours*

The values (%) are given on the fresh weight of flour, the ash values on the dry weight are shown in brackets

	Water (dried at 100°)	Ash	Nitrogen	Protein (N × 5.7)
Wheat A:				
Wholemeal	15.60	1.49 (1.77)	1.90	10.83
Straight run, untreated	16.00	0.45 (0.53)	1.73	9.86
Straight run, bleached	16.29	0.45 (0.53)	1.74	9.92
Wheat B:				
Straight run, untreated	14.58	0.48 (0.56)	1.96	11.17
Patent, untreated	15.43	0.33 (0.39)	1.82	10.37

The vitamin estimations were made with the flours as they were received, and, therefore, containing 15–16 % of water. During the experiments the flours were stored in a cool place in the dark.

Breads

In a series of preliminary experiments a sample of commercial bread was obtained weekly from a large London manufacturer and fed either in the fresh state or after drying at 37° and pulverizing. A comparison of the results showed that fresh and dried breads gave no different vitamin contents, so in all later tests dried bread crumbs, the most convenient form for handling bread, were used. This bread was made by the sponge method, which involves making approximately one-third of the flour into a soft dough with more than half the total water and all the yeast. This sponge is left to work for approximately 1½ hr. at 84° F. and the remainder of the flour and water, the salt and other ingredients are then added, thoroughly mixed and allowed to work for a further period of about 1½ hr. before making into loaves. The details of the method of manufacture of the commercial sample were not obtainable, but it was understood that it contained approximately 0.16% yeast solids to 100 lb. of flour. The water content was 33% by drying at 37° and 37.6% by drying at 102°.

The other breads examined were prepared for us under carefully controlled conditions by Dr Kent-Jones. They were made from the tested flours, wheat (A) and yeast (baker's yeast III), the white breads being made from the unbleached, straight run flour and the brown from the wholemeal. Three systems were used in making the white breads, details of which are given below; the quantities of materials employed are given in Table II.

Table II. *The composition of the various breads*

Bread	As made				% water in fresh bread dried at		% constituents in bread dried at 37°		
	Flour lb.	Yeast oz.	Salt oz.	Water lb.	37°	100°	Flour	Yeast, dry	Salt
White, sponge	—	—	—	—	33	37.6	—	0.16	—
White, short dough	25	4	5	13	31	36.0	98.44	0.28	1.28
White, long dough	25	1½	6	12½	31	36.0	98.42	0.09	1.49
White, baking powder	25	0	5*	14	23	27.8	98.75	0	1.25
Wholemeal	23	4	5½	13½	36	40.7	98.10	0.30	1.60

* In addition to NaCl this mixture contained 6 oz. acid calcium phosphate and ¼ oz. sodium bicarbonate.

Short dough process, white bread. In this system the dough only works for about 3 hr. from the time of mixing until the time of baking. The 38 lb. 9 oz. of materials yielded 17 2 lb. loaves (scaled off at 2 lb. 3 oz.), leaving 1½ lb. discard. The flour and yeast at 66° F. were mixed with the water at 94° F., giving a dough temperature of 82° F. This was knocked back after 1½ hr., moulded after 3 hr. and entered the oven 3 hr. 20 min. after mixing. It was baked for 50 min., the oven temperature being 500° F. The temperature registered on a maximum thermometer in the centre of a loaf was 99°.

Long dough process, white bread. In this system the dough works for about 10 hr. between mixing and baking. The 38 lb. of materials were again made into 17 2 lb. loaves (scaled off at 2 lb. 3 oz.), 13 oz. being discarded. The flour and yeast at 62° F. was mixed with water at 85° F., giving a dough temperature of 76° F. The dough was knocked back after 8 hr., when the temperature was 74° F., scaled off after 8 hr. 40 min., given its final moulding after 9 hr. 15 min. and put in the oven 10 hr. 15 min. after mixing. It was baked for 50 min., the oven temperature being 500° F., and the internal temperature of the loaves, as registered on a maximum thermometer, 99°.

Short dough process, wholemeal bread. The 37 lb. 5 oz. of materials yielded exactly 17 2 lb. loaves, scaled off at 2 lb. 3 oz. The times of the fermentation process were essentially the same as in the case of the short dough white bread; the dough temperature was 79° F. The bread was baked in this case for 60 min. in an oven at 500° F.

The water contents of the breads were determined both after drying at 37° as fed, and also after drying to constant weight at 100° (Table II).

Baking powder bread. The dough consisted of flour mixed with 1.25% of salt and 1.51% acid calcium phosphate and 1.07% of sodium bicarbonate, these last two materials constituting the self-raising ingredients of the dough and replacing the yeast. The mixture was placed at once in the oven and baked for 45 min., the oven temperature being 450–480° F.

METHODS

In all the vitamin tests the criterion was the growth of young rats. The rats were fed on a basal diet containing 60% rice starch, 20 casein, 15 fat and 5 normal salt mixture. The fat consisted of 4 parts of hardened cottonseed oil and 1 part of lard, the latter being included to ensure adequate supply of unsaturated fatty acids in the ration [Burr & Burr, 1930; Hume, private communication]. The diet was mixed with its own weight of water and steamed for 3–5 hr., depending on the amount made up, to dextrinize the starch and guard against refection [Fridericia *et al.* 1927]. This basal diet was supplemented with daily doses of cod liver oil and the various constituents of the vitamin B complex other than the one being tested. To this regime was added, after a preliminary period of depletion, the test material (yeast, flour or bread) and the weight increases of the rats were observed for 4 weeks and compared with those of rats of the same litters receiving the basal diet together with doses of the appropriate standard materials (see below). Each dose was tested on several rats, the sexes being evenly divided where possible.

The dried yeast doses were given as weighed daily amounts and were in all cases completely consumed. The flours and breads were, however, too bulky for this method to be applicable and they were, therefore, made into test diets containing 68% flour or dried bread crumbs, 12 casein, 15 fat and 5 salt mixture. Since the flours and therefore the breads contained 10% protein, these diets contained roughly the same amounts of protein, carbohydrate and fat as the basal diet. The test diets were not fed *ad lib.*, but a weighed quantity (5–13 g. wet weight as required) was fed daily, the remainder of the animals' requirements being made up with the basal diet. If any of the test diet was left on one day a proportionately larger amount was given the next day. In some of the experiments with wholemeal bread and flour, when very small daily amounts were needed, the necessary daily doses were given as 5 g. of diets containing 34% bread or flour, 16 casein, 30 rice starch, 15 fat and 5 salt mixture. As the test diets were also cooked with their own weight of water the actual intake of test material was calculated as 34 or 17% of the daily intake according to the type of diet. Thus 5 g. of the 68% bread diets were equivalent to a bread dose of 1.7 g. daily, and 13 g. to 4.4 g. daily and so on.

Vitamin B₁. The method employed was essentially that described by Chick & Roscoe [1929]. The rats were started on their doses of test material when their weights on the basal diet with only vitamin B₂ complex as supplement had been stationary for one week. The average body weight of the rats at the beginning of the tests was 65 g. Vitamin B₂ was given as an aqueous acid extract of yeast autoclaved for 5 hr. at 120° and pH 5.0 [Roscoe, 1930]. The daily dose of 1 ml. was equivalent to 0.5 g. of original yeast (dry weight).

Rats receiving 10 mg. daily of the international standard (activated acid clay) containing 1 international unit (I.U.) of vitamin B₁ were included in each litter and their growth was compared with that observed with rats receiving the various doses of test materials.

Vitamin B₂ complex. Since vitamin B₂ has been found to contain flavin and at least one other factor necessary for the growth of rats, it was essential to test

for these constituents separately. Tests for the whole complex were also carried out as it was thought interesting to compare them with tests of the individual constituents. The method was that of Chick & Roscoe [1928]. Vitamin B₁ was supplied as a concentrate from yeast made by the method of Peters, as modified by Kinnerley *et al.* [1933], the daily dose of 0.1 ml. being equivalent to 0.6 g. of the original yeast (dry weight). This was sufficient for normal growth when all vitamin B₂ factors were present.

Negative control rats were included in many of the litters but not in all as in every case their growth was negligible. The average weight of the rats when test materials were first given was 50 g. In each litter some rats were fed 100 or 200 mg. of baker's yeast III daily which was used as a standard of comparison in tests for vitamin B₂ complex and its separate constituents, and the growth obtained compared with that of rats receiving the test materials.

Flavin. The method of estimating flavin was similar to that for the whole vitamin B₂ complex, but, in addition to the supplement of vitamin B₁, the rats also received doses of an autoclaved yeast extract from which flavin had been removed by adsorption on fuller's earth [see Chick *et al.* 1935]. The daily dose of 1 ml. was equivalent to 0.6 g. original dry yeast.

It was assumed that this supplementary dose, which was identical with the "supplementary material" of Chick *et al.* [1935], was also identical with György's [1935] vitamin B₆, and together with flavin would be adequate to provide the entire vitamin B₂ complex. Researches by Edgar & Macrae [1937] carried on while the present work was in progress have shown that this preparation contains only one of two factors required to supplement flavin in providing the whole vitamin B₂ complex, and that a second factor is adsorbed with the flavin on the fuller's earth. The factor present in the extract which we used is termed the "filtrate factor" and that adsorbed, the "eluate factor".

It was thus possible that our tests for flavin, carried out as described above, were invalid owing to a concomitant deficiency of "eluate factor", but at the same time the results showed that all the materials tested provided excellent supplements for flavin and therefore might be presumed to contain large amounts of both "eluate" and "filtrate" factors. In every case a dose of flour or bread which promoted a certain amount of growth when given as source of flavin, promoted much better growth when given as a source of the vitamin B₂ factors other than flavin. We therefore conclude that the weight increases observed in the flavin tests cannot have been limited by deficiency of any other part of the vitamin B₂ complex.

To confirm this point an experiment was performed and is reported in Part III of this paper, in which flavin tests on white breads were made with added "filtrate factor" and also with both "eluate" and "filtrate" factors. The method of preparation of the yeast extract containing the "eluate factor" is described by Edgar & Macrae [1937] who kindly prepared these fractions for us. Doses of 1 ml. equivalent to 0.5 g. of dry yeast were given daily. The results obtained showed that growth with added "filtrate" and "eluate" factors was the same as with "filtrate" factor alone or with neither.

Since the "eluate" and "filtrate" factors together adequately supplemented flavin, it would have been possible to use flavin as a standard in these tests, but our limited knowledge at the time of planning these experiments decided us to use baker's yeast III as a standard for all tests of the constituents of the vitamin B₂ complex. Accordingly in all litters one or two positive control rats received 100 or 200 mg. daily of this yeast with the same supplements as the rats receiving the test materials. We have since attempted to determine the flavin equivalent

of the yeast, but the results obtained were not sufficiently conclusive to warrant the expression of our results in terms of the actual amounts of flavin in the materials.

Factors other than flavin. The tests for these factors were made also by a method similar to that used for the vitamin B₂ complex and for flavin, but in this case all the rats received an addition of pure crystalline hepatoflavin as well as vitamin B₁. The flavin was administered in solution, each rat receiving 12γ daily, an amount which, with adequate vitamin B₁ and other vitamin B₂ factors, was found sufficient to maintain growth of 14–16 g. weekly for 4 weeks. Control rats receiving only the basal diet with vitamin B₁ and flavin grew slightly, but the growth was very small in proportion to that obtained with rats receiving the flour and bread test doses. The baker's yeast III was used as a standard of reference in all tests. Whether "eluate factor" or "filtrate factor" was the limiting factor in the materials tested, it remains for further work to show.

I. THE B-VITAMINS IN VARIOUS YEASTS

Experimental

The results of tests for the B-vitamins in a sample of brewer's yeast and three samples of baker's yeast are given in Table III. With the exception of the baker's yeast III, which was used for making the breads, the tests were not very

Table III. *Weight increases of rats receiving yeasts as source of the constituents of the vitamin B complex*

Yeast	Dose mg. daily	Average weekly gain in weight (g.). No. of rats in brackets. Rats receiving yeast as source of			
		Vitamin B ₁	Vitamin B ₂ complex	Flavin	Vitamin B ₂ factors other than flavin
Brewer's	20	7 (4)	—	—	—
	50	24 (3)	—	—	—
	100	22 (2)	11 (4)	14 (1)	11 (1)
	200	—	15 (2)	—	—
Baker's I	100	10 (4)	10 (4)	12 (2)	10 (1)
	150	11 (2)	—	—	—
	200	—	16 (3)	—	—
Baker's II	50	12 (4)	—	—	—
	100	18 (3)	—	—	—
	200	—	11 (5)	13 (2)	15 (2)
Baker's III New	100	10 (4)	9 (4)	9 (3)	13 (5)
	200	—	12 (4)	13 (4)	16 (2)
After storage for 12 months	100	—	12 (3)	12.5 (3)	19 (2)
	150	9 (4)	—	—	—
International standard	10 (1 i.u.)	13 (8)	—	—	—

extensive. They do, however, show that considerable variations, in all B-vitamins, occur in different varieties and samples of yeast.

Vitamin B₁. The growth of the positive control rats which received 1 i.u. of vitamin B₁ daily, as 10 mg. of activated acid clay standard preparation, varied very little and averaged 13 g. per week. With this amount of weight increase as criterion, it was estimated that 1 i.u. of vitamin B₁ was present in 20–50 mg. of the brewer's yeast, in rather more than 150 mg. of baker's yeast I, in 50 mg. of baker's yeast II and in more than 100 mg. of baker's yeast III.

In the case of baker's yeast III a further test was made 12 months after the first, which had been carried out when the yeast was first received and dried. Most unexpectedly, when the yeast was fed at 150 mg. level, the growth resulting was no better than that originally obtained with 100 mg. (10 g. per week) though the control rats receiving the international standard still showed a weight increase of 13 g. weekly. It thus appeared that the vitamin B₁ in this yeast had diminished by one-third during 12 months' storage at room temperature in the dark.

Vitamin B₂ complex. The first three yeasts were tested for vitamin B₂ complex before baker's yeast III had been obtained so that litter mate controls receiving this yeast could not be included in the experiments. The results can, however, be compared with the earliest tests of baker's yeast III. The samples of brewer's yeast and baker's yeast I contained twice as much of the complex as did baker's yeasts II and III, since the rats receiving 100 mg. of the former yeasts showed the same weight increases as those which received 200 mg. of the latter. Tests for flavin and other factors were also carried out. The brewer's yeast and baker's yeast I contained about twice as much flavin as did baker's yeasts II and III, 100 mg. of each of the former and 200 mg. of each of the latter promoting equal growth. All the yeasts contained similar amounts of factors other than flavin; 100 mg. of the brewer's yeast or baker's yeast I promoted slightly less growth than did 100 mg. of baker's yeast III, and 200 mg. of baker's yeast II promoted growth about equal to that produced by 200 mg. of baker's yeast III.

Comparison of the tests for the whole complex and those for the components showed that baker's yeasts II and III were relatively deficient in flavin, which was therefore the limiting factor in their growth-promoting effect as source of the complex and that the brewer's yeast and baker's yeast I contained limiting amounts of factors other than flavin.

In Table III two estimations of the vitamin B₂ complex and its components in baker's yeast III are included, one made when the yeast was fresh and one after 12 months' keeping. The striking fact appears that the growth of the rats with the same doses was greater in the later tests than in the earlier. This illustrates a fact which became more apparent as the work proceeded, namely that the growth resulting from feeding this yeast as source of vitamin B₂ complex, flavin or factors other than flavin, gradually increased as time went on. The most likely explanation is that the rats used in the later experiments responded by greater weight increases to the same amounts of vitamins, owing to

Table IV. *The B-vitamins in various yeasts*

In brackets are given the I.U. of vitamin B₁ per g. of material. Comparison may only be made between figures in vertical columns since estimations of the separate B-vitamins were independent of each other.

Yeasts:	Amounts (mg.) promoting equal weight increases when given as source of			
	Vitamin B ₁	Vitamin B ₂ complex	Flavin	Vitamin B ₂ factors other than flavin
Brewer's	20-50 (35)	100	100	>100
Baker's I	>150 (<7)	100	100	>100
Baker's II	50 (20)	200	200	100
Baker's III	>100 (<10)	200	200	100
Standard:				
International acid clay	10 (100) (1 I.U.)	—	—	—

seasonal or other variations in their growing powers. Such variations have frequently been noted, though in this series of experiments they were surprisingly large. Since, in all experiments, tests with the respective standard materials were included for comparison, valid conclusions can still be drawn.

An attempt was made to estimate the flavin content of the standard baker's yeast III in a litter where both yeast and flavin were fed with "filtrate" and "eluate" factors. The rats receiving $4\frac{1}{2}$, 9 and 18γ of flavin daily grew averages of 12, 13 and 15.5 g. weekly, while those receiving 100 and 200 mg. of the yeast daily grew 16.5 and 18 g. weekly (2-week tests). It thus appeared that 100 mg. of yeast were equivalent to 18γ of flavin, or 1 g. would have contained 180γ .

The results of the yeast tests are summarized in Table IV.

Discussion

The brewer's yeast was the richest source of vitamin B₁, 1 g. containing 20–50 I.U. (35 I.U. approx.). Baker's yeast II was nearly as good, 1 g. containing 20 I.U., but this yeast had been specially "reinforced" with vitamin B₁, the method of reinforcement not being known. The two ordinary baker's yeasts I and III were much poorer sources of the vitamin, containing less than 7 I.U. and less than 10 I.U. per g., respectively. Baker's yeast III was found, after storage for 12 months in a well-stoppered bottle at room temperature in the dark, to have diminished in vitamin B₁ activity by about 30%.

Not many comparisons have been made between yeasts and the international standard, but Chick & Jackson [1932] and Birch & Harris [1934] tested the same brewer's yeast by various methods and found values varying from 17 to 20 I.U. per g. dry weight. Baker & Wright [1935] found two brewer's yeasts to contain 6 and 23 I.U. per g., respectively, and Lassen [1936] found top-fermenting brewer's yeasts to have an average value of 12.1 I.U. per g., bottom-fermenting brewer's yeasts 36.1 I.U. and distiller's yeast 6.6 I.U. These values are of the same order as those reported here. The superiority of brewer's over baker's yeasts was shown by Williams [1921] and Plimmer *et al.* [1927], who found the former the better source of "vitamin B". Scheunert & Schieblich [1929] and Quinn *et al.* [1930] found more vitamin B₁ in brewer's yeast than in baker's yeast. Drummond & Whitmarsh [1932] found more of both vitamin B₁ and vitamin B₂ in several samples of brewer's yeast than in several samples of distiller's yeast, but Lassen [1936] found that brewer's yeast was poorer than baker's yeast in vitamin B₂.

Our tests for flavin and other factors of the vitamin B₂ complex suggested that baker's yeasts II and III contained about half as much flavin and rather more of the other factors than the brewer's yeast and baker's yeast I. The variations were less marked than those in the vitamin B₁ content. This is again in agreement with the work of Quinn *et al.* [1930] who found that two brewer's yeasts promoted equal growth when given as source of vitamin B₂. In their tests an alcoholic extract of wheat was fed as source of vitamin B₁ and this would probably have contained all B-vitamins other than flavin, so that these tests may be taken as tests for flavin.

The flavin in yeasts has also been estimated spectrophotometrically. Using this method Warburg & Christian [1933] found that a baker's and a brewer's yeast contained similar amounts of flavin, 36 and 30γ per g. of dry yeast, respectively, and values of 18 and 18–21 γ per g. were found by Kuhn *et al.* [1934] and Euler & Adler [1934], in both cases for dried brewer's yeasts. These figures are about one-tenth of those found by us in a biological test; from which it was calculated that 1 g. of dried baker's yeast contained 180γ of flavin. Until this

discrepancy can be explained, it seems misleading to calculate from our figures the actual flavin contents of the flours and breads, which were compared with the yeast.

The possible significance of these variations for human nutrition will be considered in the later section dealing with breads.

II. THE B-VITAMINS IN VARIOUS FLOURS

Experimental

Vitamin B₁. Table V shows the results of the tests for vitamin B₁ in the bleached and unbleached straight run flours and the wholemeal flour from wheat A, and of straight run and patent flour from wheat B.

Table V. *Weight increases of rats receiving flour as source of vitamin B₁*

Wheat A: Flour	Daily consumption g.	Average weekly gain in weight (g.). No. of rats in brackets		Litter nos.
		Rats receiving flour	Rats receiving standard*	
White, straight run, unbleached				
New	2.7	13 (5)	15 (2)	66, 71
	5.2	20 (4)	14 (2)	61, 62
12 months old	2.7	12 (2)	13.5 (1)	106
White, straight run, bleached				
New	2.7	12 (5)	15 (2)	66, 71
	5.2	17 (5)	14 (2)	61, 62
12 months old	2.7	14 (2)	13.5 (1)	106
Wholemeal	0.85	10 (3)	7 (2)	110
	1.4	17 (2)	13 (1)	102
	1.7	14 (3)	0 (1)†	90
	1.7	22 (3)	12 (1)	94
	2.7	27 (2)	—	87
Wheat B:				
White, straight run, unbleached	2.7	11 (3)	9 (2)	118
White, patent, unbleached	2.7	-1 (3)‡	9 (2)	118

* The standard fed was 10 mg. of activated acid clay, containing 1 I.U. of vitamin B₁.

† A very poor litter, the rat receiving standard did not grow at all.

‡ These rats lost weight and developed polyneuritis after 3 weeks.

Wheat A. Of the unbleached white flour 2.7 g. promoted slightly less growth than did 10 mg. of the acid clay standard; this amount therefore contained about 1 I.U. of vitamin B₁. The rats receiving the bleached flour grew less well, but the difference was very slight. These flours were tested again after storage for 12 months in the dark in a cool place. Rats receiving 2.7 g. daily of either flour now grew as well as the controls receiving the dose of the standard. The vitamin B₁ in the flours had not therefore diminished during storage.

The wholemeal flour was a good source of vitamin B₁, 1 I.U. being present in about 0.85 g. of the flour.

Wheat B. This sample of straight run, unbleached white flour contained about the same amount of vitamin B₁ as that derived from wheat A. 2.7 g. daily promoted rather better growth than 10 mg. of the standard, so that this amount contained 1 I.U. But the sample of top patent flour tested not only

failed to promote growth as a source of vitamin B₁, but the three rats which were eating 2.7 g. daily of the flour developed polyneuritis within 3 weeks. The symptoms were cured in each case by changing the ration of patent flour to an equal one of the straight run white flour from the same wheat, B.

Vitamin B₂ complex (Table VI). In these and the tests for the separate constituents of the vitamin B₂ complex only the flours of wheat A were tested.

Table VI. *Weight increases of rats receiving flour as source of the vitamin B₂ complex*

Flour (Wheat A)	Daily con- sumption g.	Average weekly gain in weight (g.). No. of rats in brackets				Litter nos.
		Control rats	Rats receiving flour	Rats receiving standard*		
				100 mg.	200 mg.	
White, straight run, unbleached	4.3	0 (2)	12 (6)	8 (2)	12.5 (2)	63, 64, 68, 69
White, straight run, bleached	4.3	0 (2)	10 (6)	8 (2)	12.5 (2)	63, 64, 68, 70
Wholemeal	2.0	—	11 (4)	13 (2)	—	101
	2.1	0 (1)	10 (5)	—	12.5 (1)	88, 89
	4.5	2 (1)	14 (3)	—	13 (1)	84

* The standard fed was baker's yeast III.

4.3 g. daily of the unbleached white flour promoted growth equal to that resulting when 200 mg. of baker's yeast III were fed. The same amount of the bleached flour promoted slightly less growth, and 4.5 g. of the wholemeal flour were found to be equivalent to 200 mg. of the standard yeast. There was thus little difference between the activities of wholemeal and straight run white flour when providing the source of the whole vitamin B₂ complex.

Table VII. *Weight increases of rats receiving flour as source of flavin*

Flour (Wheat A)	Daily con- sumption g.	Average weekly gain in weight (g.). No. of rats in brackets				Litter nos.
		Con- trol rats	Rats receiving flour	Rats receiving standard*		
				100 mg.	200 mg.	
White, straight run, unbleached						
New	3.2	1 (2)	6 (3)	—	10 (1)	73, 79
	3.5	3 (2)	8.5 (4)	—	12 (3)	74, 79
	4.3	5 (1)	12 (2)	—	—	69, 70
9 months old	3.3	2 (2)	9 (6)	9 (2)	16 (2)	96, 97
White, straight run, bleached						
New	3.7	3 (2)	7.5 (2)	—	12 (3)	74, 79
	4.2	3 (2)	9 (3)	—	—	69, 70, 73
9 months old	3.1	2 (2)	4 (3)	9 (2)	16 (2)	96, 97
	4.2	2 (1)	12 (1)	8 (1)	16 (1)	97
Wholemeal						
9 months old	2.0	—	12 (3)	12.5 (2)	—	98
	3.1	4 (1)	10 (5)	—	17.5 (1)	89, 91

* The standard fed was baker's yeast III.

Flavin (Table VII). 4.3 g. daily of the unbleached flour and 200 mg. of baker's yeast III promoted equal growth (12 g. weekly) when supplying flavin.

But the rats receiving 4.3 g. daily of the bleached flour did not grow so well, showing that the flavin in the flour was to some extent destroyed by the bleaching process. In no case was sufficient of the bleached flour eaten by the rats to supply as much flavin as was contained in 200 mg. of the standard yeast, but from a comparison of the figures which show that 4.2 g. of bleached flour contained as much flavin as 3.4 g. of the unbleached, and that 3.7 g. of bleached contained as much as 3.2 g. of unbleached, it may be calculated that about 5.0–5.3 g. of bleached would have contained as much as 4.3 g. of unbleached or 200 mg. of yeast. The bleaching process had thus reduced the flavin content of the flour by 15–20 %.

The rats receiving the unbleached flour after storage for 9 months grew to the same extent as those receiving equal amounts before storage, but the growth of controls receiving the standard must be taken into account and it is then obvious that all the animals were growing more vigorously at the time of the second test. In fact, in the later experiments, 3.3 g. of the stored flour and 100 mg. of the yeast promoted equal growth as source of flavin, so that 6.6 g. of the flour would have been equivalent to 200 mg. of the yeast. In the earlier test 4.3 g. had been equivalent to 200 mg. of yeast so that the flour may be estimated to have lost 35 % of its flavin during keeping in a cool, dark store for 9 months.

Similarly, 3.1–4.2 g. of the stored, bleached flour had the same activity as 100 mg. of yeast, so that 6.2–8.4 g. were equivalent to 5.0–5.3 g. of the fresh material, and the bleached flour had lost a further 20–40 % of its flavin content. Thus, after keeping, the bleached flour still contained less flavin than the unbleached, but the difference was not now so marked.

Of the wholemeal flour 2.0 g. daily were equivalent in activity to 100 mg. baker's yeast III, that is 4.0 g. were equivalent to 200 mg.

Vitamin B₂ factors other than flavin (Table VIII). In the first tests 4.3 g. daily of either the unbleached or the bleached white flours supplemented with

Table VIII. *Weight increases of rats receiving flour as source of vitamin B₂ factors other than flavin*

Flour (Wheat A)	Daily con- sumption g.	Average weekly gain in weight (g.). No. of rats in brackets				Litter nos.
		Con- trol rats	Rats receiving flour	Rats receiving standard*		
				100 mg.	200 mg.	
White, straight run, unbleached						
New	4.3	3 (2)	13 (4)	—	16 (2)	68, 70, 72, 73
11 months old	4.3	8 (1)	21 (3)	17 (2)	—	99
18 months old	1.7	—	12 (3)	13 (1)	—	114
White, straight run, bleached						
New	4.3	3 (2)	13 (5)	—	16 (2)	70, 72, 73
11 months old	4.3	8 (1)	21 (2)	17 (2)	—	99
Wholemeal	0.85	6 (1)	12 (3)	18 (1)	—	115
	1.2	3 (1)	18 (2)	16.5 (2)	—	103
	1.7	7 (3)	19 (6)	16 (3)	—	93, 105
	3.1	—	22 (2)	13.5 (1)	—	89

* The standard fed was baker's yeast III.

flavin, promoted equal growth of 13 g. per week, while 200 mg. of baker's yeast III produced growth of 16 g. weekly. These factors did not therefore

appear to have been affected by bleaching. The flours were tested again after storage for 11 months and were still found to have equal activity, 4.3 g. daily of either promoting a growth of 21 g. weekly, whilst 100 mg. of the yeast promoted growth of 17 g. Seven months later a further test was made of the unbleached flour at a lower level of intake, 1.7 g. daily, producing growth of 12 g. weekly which was comparable with that obtained with 100 mg. of the yeast, tested simultaneously, 13 g. weekly. Since in the first tests 4.3 g. of flour contained rather less of the factors than did 200 mg. of yeast, that is 2.1 g. would have had less activity than 100 mg., and in the last experiments 1.7 g. of the unbleached flour were equal in activity to 100 mg., it does not appear that there had been any loss of activity during storage for 18 months.

In these tests the activity of 1.2 g. of wholemeal was found to be slightly greater and that of 0.85 g. slightly less than that of 100 mg. of baker's yeast III.

The results of all the tests with the flours are summarized in Table IX. From the similarity of the amounts needed for growth as source of the vitamin B₂

Table IX. *The B-vitamins in various flours*

In brackets are given the I.U. per g. of material. Comparison may only be made between figures in vertical columns since estimations of the separate B-vitamins were independent of each other.

		Amounts (g.) promoting equal weight increases when given as source of			
		Vitamin B ₁	Vitamin B ₂ complex	Flavin	Vitamin B ₂ factors other than flavin
Wheat A:	Flour				
	White, straight run, unbleached				
	New	2.7 (0.37)	4.3	4.3	>2.1
	9-12 months old	2.7 (0.37)	—	6.6	<4.3
	18 months old	—	—	—	1.7
	White, straight run, bleached				
	New	2.7 (0.37)	>4.3	5.0-5.3	>2.1
	9-12 months old	2.7 (0.37)	—	6.2-8.4	<4.3
	Wholemeal	0.85 (1.18)	4.5	4.0	0.85-1.2
Wheat B:					
	White, straight run, unbleached	2.7 (0.37)	—	—	—
	White, patent, unbleached	— (0)	—	—	—
Standards:					
	International acid clay	0.01 (100)	—	—	—
		(1 I.U.)			
	Baker's yeast III	—	0.2	0.2	0.1

complex and of flavin respectively, and the considerably smaller amounts required as source of factors other than flavin, it is obvious that in the tests for the whole complex the deficiency of the flavin component is the limiting factor.

Discussion

(1) *The B-vitamins in whole wheat.*

The wholemeal from the sample of wheat tested in this work contained 1 I.U. of vitamin B₁ in 0.85 g., or 1.18 I.U. in 1 g. This value is lower than those recorded by other workers, who, however, found great variations in different samples. Baker & Wright [1935] obtained values of 2.3, 2.6 and 3.4 I.U. per g.; Scheunert & Schieblich [1936] 1.2, 1.5 and 1.5 I.U. per g.; Baker *et al.* [1937]

1.2, 2.0, 2.1, 2.3, 2.5 and 2.6 I.U. per g. and Leong & Harris [1937] 1.5 I.U. per g.

Until international standards for flavin and the other constituents of the vitamin B₂ complex have been set up, it will be impossible to compare estimations of these factors made by different workers, and indeed few have been made.

A comparison of the potencies of whole wheat as source of the different B-vitamins showed clearly that to promote an equal amount of growth much less, about one-fifth, was needed to supply vitamin B₁ than to supply the vitamin B₂ complex, and that the amount of flavin present was the limiting factor in the vitamin B₂ complex (see Table IX). This fact has already been pointed out by Birch *et al.* [1935] and Copping [1936]. The conclusion, however, would not be warranted that wheat would be for all mammals a better source of vitamin B₁ than of vitamin B₂ complex, and a less good source of flavin than of other factors of the vitamin B₂ complex, for comparison of the relative amounts needed to promote a certain submaximum growth effect is not necessarily a criterion of the relative amounts needed to supply adequately the complete demands of even the same species. Moreover, different species may have different requirements.

(2) *The effect of milling on the B-vitamins in wheat.*

Vitamin B₁. The wholemeal flour contained 1.18 I.U. of vitamin B₁ per g., and the straight run flour milled from the same wheat (A) 0.37 I.U. per g., or one-third as much. The straight run flour from wheat B also contained 0.37 I.U. per g. but the corresponding patent flour contained no appreciable amounts of this vitamin. Since the straight run flours represented 70–72 % of the whole grain, it may be calculated that about 20 % or one-fifth of the vitamin in the whole grain was present in these flours. On the other hand the patent flour which represented 42 % of the whole grain contained negligible amounts of the vitamin. Since it has been shown [see Kent-Jones, 1934, p. 19] that the ash of pure endosperm is very constantly 0.30 % and that of the husk (all outer coverings and germ) 8.00 % it may be calculated that the straight run flours contained 2.34 % of the husk of the whole grain whilst the patent flour contained only 0.45 %. It thus seems probable that vitamin B₁ is present only in the husk, and that any present in the white flour is due to small amounts of the outer coverings of the grain present. The vitamin content does not exactly parallel the husk content, but this is no doubt due to the fact that the different covering layers vary in vitamin content and are not equally represented in the husk which passes into the white flour.

Chick & Hume [1917] could find no evidence for the presence of vitamin B₁ in a straight run flour; the pigeons with which they worked developed polyneuritis as quickly as those receiving polished rice. In order to control the present method which uses the growth of rats as criterion, we compared our straight run flour with polished rice. Whilst the rats receiving polished ground rice gained no weight those receiving equal amounts of straight run flour grew well. One can therefore only conclude that the sample of straight run flour examined by Chick & Hume was a more highly milled product than ours.

Bell & Mendel [1922] found that although mice would not grow when patent flour was the sole source of the vitamin B complex, they grew fairly well when "clear" flour was fed and very well when low grade white flour was fed. Vitamin B₁ must therefore have been present in the "clear" and low grade white flours. More recently Scheunert [1936] has shown that a flour of 60 % extraction, that is a "patent" flour, contained no appreciable vitamin B₁, but that flours of 75 and 82 % extraction contained 40 and 60 % respectively of the vitamin B₁ of

the whole grain. In a later paper [Scheunert & Schiebllich, 1937] the following absolute values are given: whole wheat 1.5 I.U. per g.; flours of 94, 82, 75 and 60 % extraction 1.2, 1.0, 0.4 and 0.24 I.U. per g. respectively. Leong & Harris [1937] tested various parts of grain, not, however, derived from the same sample of wheat; two white flours contained 0.3 and 0.2 I.U. per g. With these figures may be compared our value of 0.37 I.U. per g. for a flour of 70 % extraction.

Flavin. A comparison of the tests for this factor in wholemeal and straight run flours made at the same time, that is after keeping for 9 months, showed that 4.0 g. of the wholemeal were equivalent to 6.6 g. of the straight run unbleached flour or 200 mg. standard yeast. Thus the wholemeal contained $1\frac{1}{2}$ times as much flavin as the white flour and the latter contained 42 % of the flavin in the whole grain.

Copping [1936] found that whole wheat contained more flavin than did white flour, but a quantitative estimation was not possible. Scheunert & Schiebllich [1937] tested flours and whole wheat and found that the latter contained five times as much vitamin B₂ as flours of 75 % or lower extraction, but only $1\frac{1}{2}$ times as much as flours of 82 and 94 % extraction. This test was probably a test for flavin.

Vitamin B₂ factors other than flavin. In respect of these factors the wholemeal flour was richer than the straight run white flours, about 1.0 g. of the former and 1.7 g. of the latter being equivalent to 100 mg. of the standard yeast. The relative distribution was about the same as in the case of flavin; the white flour contained about 40 % of the activity of the whole grain. This result is in agreement with the finding of Copping [1936], who considered that the whole grain contained about three times as much of the factors required to supplement flavin as did white flour. In her experiments, however, the two materials were not obtained from the same sample of wheat, and the white flour was a patent, not a straight run, flour.

(3) *The effect of bleaching and keeping on the B-vitamins in flour.*

The bleaching agent employed for the sample of flour investigated was nitrogen peroxide. The effects observed cannot be assumed also for other bleaching agents except in so far as they may be due to oxidation. It is also possible that variations in the details of the process, even with the same agent, might be important. The effect of storage on the flour may profitably be considered at the same time, since in general bleaching may be said to hasten processes which occur naturally in time. For general reviews of bleaching and its effects Hamill [1911] and Kent-Jones [1933] should be consulted.

Our results suggested that the vitamin B₁ in white flour was very slightly reduced by the bleaching process, but the difference between the bleached and the unbleached flours was so slight that further work would be necessary to establish this with certainty. No deterioration in the vitamin B₁ content of either flour was apparent after keeping for 12 months. Thus neither bleaching nor keeping had a significant effect on the vitamin B₁ content of white flour.

The flavin content of the flour was definitely affected by bleaching and by keeping. This is not surprising in view of the fact that flavin is unstable to light or oxidation, readily yielding colourless compounds which are biologically inactive. The bleaching process was found to have reduced the flavin content of the flour by 15–20 % and this flour lost a further 20–40 % of its flavin during 9 months' storage, so that at the end of 9 months the bleached flour still contained less flavin than the unbleached. If the flavin content of the unbleached flour be taken as 100, that of the bleached would be about 86, and of the bleached,

after keeping for 9 months, about 60, while that of the unbleached, after keeping for 9 months, would be 65.

Vitamin B₂ factors other than flavin appeared to be unaffected by bleaching or keeping of the flour, no difference being found in this respect between bleached or unbleached flours and the same flours after storage for 11 months.

Although white flour cannot be considered to be a good source of the constituents of the vitamin B₂ complex, especially of flavin, the amounts present may have a dietary significance in a restricted diet when large amounts of bread are eaten. In such cases a reduction of the flavin content by bleaching or keeping, as has been here demonstrated, might be of nutritional importance.

III. THE B-VITAMINS IN VARIOUS BREADS

Experimental

Vitamin B₁ (Table X). The commercial white bread, made by the sponge system, contained 1 I.U. of vitamin B₁ in 3.9 g. (dry weight) and the long and short dough breads, made from the white flour tested above, each contained 1 I.U. in 2.7 g.

Table X. *Weight increases of rats receiving breads as source of vitamin B₁*

Bread	Daily consumption (g.) dry	Average weekly gain in weight (g.). No. of rats in brackets		Litter nos.
		Rats receiving bread	Rats receiving standard*	
White, sponge	3.9	12 (4)	—	51
	5.2	18 (4)	—	51
White, short dough	2.7	12 (5)	13 (2)	76, 82
	2.7	9 (2)	8 (2)	117
White, long dough	2.7	12 (4)	13 (2)	76, 82
White, baking powder	2.7	0 (3)†	8 (2)	117
Wholemeal	0.85	9 (3)	7 (2)	110
	1.4	19 (2)	13 (1)	102
	1.7	15 (4)	0 (1)‡	90
	1.7	21 (3)	12 (2)	94

* The standard fed was 10 mg. of activated acid clay, containing 1 I.U. of vitamin B₁.

† These rats all developed polyneuritis within 3 weeks.

‡ A very poor litter, the rat receiving standard did not grow at all.

In the sample of white bread made with no yeast but with a self-raising mixture of acid calcium phosphate and sodium bicarbonate there was no detectable vitamin B₁; all 3 rats receiving 2.7 g. daily of this material developed polyneuritis. This material was not examined for the vitamin B₂ complex or its constituents.

The wholemeal bread was about three times as rich in the vitamin as the white breads tested, 1 I.U. being present in approximately 1 g. of dry bread.

The corresponding amounts of fresh bread containing 1 I.U. of vitamin B₁ would be 5.8 g. of the sponge process bread, 3.9 g. of each of the white breads made from the tested flour and 1.3 g. of the wholemeal bread.

Vitamin B₂ complex (Table XI). In the tests for vitamin B₂ there was difficulty in persuading the rats to eat enough of the white breads. Of these materials 4 g. had lower potency than 200 mg. of standard yeast III and 3.0 g. lower than 100 mg. of yeast. Thus more than 6.0 g. would have been required to promote the same growth as 200 mg. of yeast. There was no great difference

Table XI. *Weight increases of rats receiving breads as source of the vitamin B₂ complex*

Bread	Daily consumption g., dry	Average weekly gain in weight (g.). No. of rats in brackets				Litter nos.
		Con- trol rats	Rats receiving bread	Rats receiving standard*		
				100 mg.	200 mg.	
White, sponge	3.5	0 (1)	8 (4)	—	—	50
	4.3	0 (1)	10 (4)	—	—	50
White, short dough	3.0	2 (2)	8 (8)	9 (2)	12 (2)	75, 77, 115, 116
	3.0	—	8 (2)	15 (2)	—	104
	3.9	3 (1)	8.5 (5)	—	14 (1)	80, 83
White, long dough	3.0	2 (2)	5 (8)	9 (2)	12 (2)	75, 77, 115, 116
	3.0	—	10 (2)	15 (2)	—	104
	4.0	3 (1)	8.5 (4)	—	14 (1)	81, 83
Wholemeal	2.0	—	12 (4)	14 (4)	—	101, 104
	3.1	0 (1)	12 (5)	—	12.5 (1)	88, 92
	4.5	2 (1)	15 (3)	—	13 (1)	84

* The standard fed was baker's yeast III.

between the vitamin B₂ complex contents of the long and short process white breads, and the sponge process bread. The wholemeal bread was somewhat richer in the vitamin B₂ complex, 3.0–4.5 g. daily of the dry bread being equivalent to 200 mg. of the standard yeast.

Flavin (Table XII). Of the short dough white bread 3.9 g. had less flavin activity than 200 mg. of standard yeast, and 3.0 g. had less than 100 mg. of

Table XII. *Weight increases of rats receiving breads as source of flavin*

Bread	Daily consumption (g.) dry	Average weekly gain in weight (g.). No. of rats in brackets				Litter nos.
		Control rats	Rats receiving bread	Rats receiving standard*		
				100 mg.	200 mg.	
White, sponge	5.1	—	7† (2)	—	—	50
White, short dough	3.0	4 (1)	7 (3)	—	16 (1)	86
	3.0	3 (1)	9 (6)	11.5 (4)	—	108, 113, 116
	3.9	—	12 (2)	—	15 (1)	80
White, long dough	2.8	4 (1)	6 (3)	—	16 (1)	86
	3.0	3 (1)	6.5 (6)	11.5 (4)	—	108, 113, 116
	4.1	—	11 (2)	—	13 (1)	81
Wholemeal	2.0	—	13 (3)	12.5 (2)	—	98
	3.1	4 (1)	13 (5)	—	17 (2)	91, 92

* The standard fed was baker's yeast III.

† This test was made after a test for the whole vitamin B₂ complex in the bread, and the rats were much larger than in the other tests.

yeast. The long dough white bread promoted slightly less growth at each level of intake. Of the wholemeal bread 2.0 g. had flavin activity equal to 100 mg. of the standard yeast, and 3.1 g. rather less than 200 mg. of yeast.

An additional test for flavin in white bread was made with daily doses of 3.0 g. supplemented with the "eluate" as well as the "filtrate" constituent of vitamin B₂. When the short process bread was fed to supplement a diet containing no part of the vitamin B₂ complex the average growth of the rats was

8 g. weekly, when "filtrate" factor was fed in addition the growth was 7 g. weekly, and when both "filtrate" and "eluate" were given it was 10 g. weekly. Similar groups of rats with the long process white bread showed average growths of 5, 6 and 6 g. weekly, and those receiving the standard yeast (100 mg.) 9, 11 and 12 g. weekly. The addition of the "eluate" factor did not therefore consistently produce improved growth, so that the tests for flavin in breads and yeasts as carried out above, where vitamin B₁ and the "filtrate" factor alone were supplied, may be taken as trustworthy flavin estimations.

Vitamin B₂ factors other than flavin (Table XIII). Of both the short and long dough breads, 4.3 and 3.0 g. daily had greater effect than 100 mg. of the standard

Table XIII. *Weight increases of rats receiving bread as source of vitamin B₂ factors other than flavin*

Bread	Daily consumption (g.) dry	Average weekly gain in weight (g.). No. of rats in brackets			Litter nos.
		Control rats	Rats receiving bread	Rats receiving standard* 100 mg.	
White, sponge	6.0	—	16† (4)	—	50
White, short dough	1.7	—	13 (2)	13 (1)	114
	1.7	8 (1)	20 (3)	19 (1)	107
	3.0	5.5 (2)	18 (5)	15 (2)	85, 109
	4.3	—	17 (2)	13 (1)	80
White, long dough	1.7	—	13 (2)	13 (1)	114
	1.7	8 (1)	18 (3)	19 (1)	107
	3.0	5.5 (2)	17 (5)	15 (2)	85, 109
	4.3	—	18 (2)	13 (1)	81
Wholemeal	0.85	6 (1)	14 (3)	18 (1)	115
	1.2	3 (1)	18 (2)	16.5 (2)	103
	1.7	7 (3)	20 (5)	16 (3)	93, 105
	3.1	—	24 (2)	16 (1)	92

* The standard fed was baker's yeast III.

† This test was made after a test for the whole vitamin B₂ complex in the breads and the rats were much larger than in the other tests.

yeast, and about 1.7 g. had equal effect. Of the wholemeal bread daily doses of 3.1, 1.7 or 1.2 g. all promoted better, but 0.85 g. less good, growth than 100 mg. of standard yeast. About 1.0 g. of this bread was therefore equivalent to 100 mg. of baker's yeast III.

Thus all the breads contained relatively greater amounts of these factors than of flavin. An amount of 3.0 g. daily of the short process white bread produced, when fed as source of vitamin B₂ complex, flavin and factors other than flavin, 8, 7 and 18 g. weekly, respectively, and the same amount of the long process bread, 5.5, 7.5 and 17 g. weekly, respectively, while 3.0 g. of the wholemeal bread produced 14, 13 and 24 g. weekly, respectively. A similar result was shown in the incomplete tests of the sponge process white bread. In a group of comparable rats (litter 50) used after a preliminary test for the vitamin B₂ complex and therefore of larger body weight (85 g.) than usual at the beginning of the test, the growth when the bread was fed as source of the whole complex or of flavin was approximately the same and was much increased when the bread was fed as source of factors other than flavin.

The results of all the tests of the breads are summarized in Table XIV.

Discussion(1) *The B-vitamins in the various breads.*

Vitamin B₁. The first white bread made by the commercial sponge process contained 1 I.U. of vitamin B₁ in 3.9 g. or 0.25 I.U. per g. and the other two

Table XIV. *The B-vitamins in various breads*

In brackets are given the I.U. of vitamin B₁ per g. of material. Comparison may only be made between figures in vertical columns since estimations of the separate B-vitamins were independent of each other.

Breads	Amounts (g. dry weight) promoting equal weight increases when given as source of			
	Vitamin B ₁	Vitamin B ₂ complex	Flavin	Factors other than flavin
White, sponge	3.9 (0.25)	> 4.3	—	—
White, short dough	2.7 (0.37)	> 6.0	> 6.0	1.7
White, long dough	2.7 (0.37)	> 6.0	> 6.0	1.7
Wholemeal	0.85 (1.18)	3.1–4.5	4.0	0.85–1.2
Standards:				
International acid clay	0.01 (100) (1 I.U.)	—	—	—
Baker's yeast III	—	0.2	0.2	0.1

breads were equal in vitamin B₁ content; 2.7 g. of either contained 1 I.U., or 0.37 I.U. per g. There was thus no apparent difference between the two white breads made from the same materials by different methods.

The wholemeal bread contained 1 I.U. of vitamin B₁ in 0.85 g. so that 1 g. contained 1.18 I.U. or three times as much as the white breads made from a flour consisting of 72 % of the same wheat.

The above values represent the vitamin B₁ contents in air-dried breads. If the fresh weights of the breads are considered, the amounts per g. are as follows: white sponge process bread 0.17 I.U.; long or short process white bread 0.25 I.U.; wholemeal bread 0.75 I.U. With these values may be compared the following values obtained by other workers for the vitamin B₁ in breads, where the results have been given in terms of I.U. per g. of fresh bread: Morgan & Frederick [1935] white 0.3; whole wheat 1.3; Scheunert [1936] white 0.20–0.86; wholemeal 1.07; De Caro [1936] white, less than 0.8; whole wheat, more than 0.8; Scheunert & Schieblich [1937] white 0.16–0.67; wholemeal 0.69; Harris [1937] white 0.12, 0.15; wholemeal 0.75–0.9; Baker *et al.* [1937] white 0.14–0.20; wholemeal 1.10–1.15. These values agree well with those reported here.

It is not proposed to give a summary of the vitamin B₁ tests of bread where no standard of comparison was included or of the large amount of work which has been carried out on the "B-vitamins" in bread without differentiation of the constituents of the complex.

Vitamin B₂ and its constituents. In Table XIV it is shown that more than 6.0 g. of either the short or long process white bread and 4.0 g. of the wholemeal bread and 200 mg. of the standard yeast all contained equal amounts of flavin. There was a slight difference between the flavin contents of the two white breads, the long process bread being slightly poorer. The difference was apparent in each of the tests for this factor and would appear to be significant. It may be due to the smaller amount of yeast present in the long dough sample.

The wholemeal bread contained over 50 % more flavin than the short process white bread.

The tests for factors other than flavin in the breads showed (Table XIV) that 1.7 g. dry weight of the long and short process white breads, about 1 g. of the wholemeal bread and 100 mg. of the standard yeast contained equal amounts of these factors. Thus the wholemeal bread contained 50 % more of these factors than the two white breads which had equal contents.

(2) *The B-vitamins in bread in relation to its constituents.*

Breads made with yeast. Table XV summarizes the results of our estimations of the B-vitamins in unbleached flour and wholemeal flour (wheat sample A) and baker's yeast III, and in the three breads made from them. The value taken

Table XV. *The vitamin contents of the breads and of their constituents*

Bread	Amounts of yeast (g.) per 100 g. flour	Units of vitamin per g.		Vitamin units per 100 g. of bread			Ratio (a)/(b)
		Yeast	Flour	From yeast (a)	From flour (b)	Bread (c)	
Vitamin B ₁ . International units							
White, short process	0.28	10	0.37	2.8	37	37	1/13
White, long process	0.09	10	0.37	0.9	37	37	1/41
Wholemeal	0.30	10	1.18	3.0	118	118	1/39
Flavin. Arbitrary units. 1 unit=amount present in 100 mg. standard yeast							
White, short process	0.28	5	0.15	1.4	15	<17	1/11
White, long process	0.09	5	0.15	0.45	15	<17	1/33
Wholemeal	0.30	5	0.25	1.5	25	25	1/17
Vitamin B ₂ factors other than flavin. Arbitrary units. 1 unit=amount present in 100 mg. standard yeast							
White, short process	0.28	10	0.59	2.8	59	59	1/22
White, long process	0.09	10	0.59	0.9	59	59	1/66
Wholemeal	0.30	10	1.00	3.0	100	100	1/33

for flavin in flour was that obtained at the same time as the bread was tested, that is after 9 months' keeping. In column (b) are shown the amounts of the vitamins in the flour which went to make up 100 g. of bread (dry weight), and in column (c) the amounts in 100 g. (dry weight) of the breads. In each of the breads the amounts of vitamin B₁ and vitamin B₂ factors other than flavin could all be accounted for by the quantities supplied by the flour, and this was also true of the flavin in the wholemeal bread. An exact comparison of the flavin in the two white breads and the flour was not obtained, but the results indicated a similar conclusion.

It does not, therefore, seem that yeast had contributed any significant amounts of the B-vitamins to the breads, which is not surprising when it is seen what a small amount of yeast, in proportion to the flour, was present. Nor is it surprising that little difference should be found between the two breads containing different amounts of yeast. The only difference found was in the flavin contents of the white breads, which contained 0.28 and 0.09 parts of dry yeast per 100 parts of flour, respectively. The bread containing most yeast contained slightly more flavin. It was perhaps significant that this, the only apparent difference, occurred where the proportion of the vitamin supplied by the yeast to that supplied by the flour was higher than in any other case (see Table XV, last column).

The nutritional importance of the yeast could only have been significant if the yeast had multiplied considerably during the fermentation of the bread. Recently it has been shown that some multiplication does occur. Simpson [1936]

found that if the yeast did not constitute more than 2% of the weight of the dry ingredients a 100 or 200% increase might occur, but a 50% increase was more usual. The greatest proportion of fresh yeast in our breads was 1% but our results do not give evidence of any increase even in the long dough bread where the total fermentation time was 10 hr. 15 min., and indeed the maximum increase of 200% would scarcely be appreciable, so small were the original amounts of yeast.

The similarity of the vitamin contents of the breads and the flours from which they were baked also suggests that there was no appreciable destruction of the vitamins during baking. This was only to be expected, as the internal temperature of the loaves did not rise above 100° and the pH of dough is in the range of 5.5–5.9 at which acid reaction even the heat-labile vitamin B₁ would only suffer slow destruction.

The finding that the addition of yeast and the baking process did not materially affect the content of the B-vitamins in bread agrees with the observation of De Caro [1936], who also found that wheat and rye flours and the breads baked from them gave almost identical values for the vitamin B complex content. In his experiments vitamin B₁ was considered the limiting factor. Morgan & Frederick [1935] and Scheunert & Schieblich [1937] also found no change in the vitamin B₁ content of bread during baking.

Baking powder bread. This sample of bread, made from unbleached, straight run, white flour with baking powder instead of yeast as raising agent, contained no demonstrable amounts of vitamin B₁. The rats receiving this bread in doses of 2.7 g. daily all developed polyneuritic symptoms within 21 days and the symptoms were cured and growth restored by substituting an equal amount of a flour of equal vitamin B₁ value to that from which the bread was made. The rats lost weight while they were receiving the bread and gained an average of 14 g. weekly when the flour was given. In this case, it would appear that there had been destruction of the vitamin B₁ in the baking process when a mixture of acid calcium phosphate and sodium bicarbonate was used instead of yeast. The pH of the dough of a similar baking powder bread was found by Dr Kent-Jones to be 6.9–7.2. This is considerably higher than that of bread made with yeast (pH 5.5–5.9) and it is possible that 45 min. heating at 100° in this less acid medium might destroy some of the vitamin B₁. It must, however, be noted that this bread was made from flour which had been stored for 18 months and which may have deteriorated, although no diminution in vitamin B₁ value had occurred after storage for 12 months.

The low vitamin B₁ content found in baking powder bread is interesting and suggestive. The result was, however, obtained from one small experiment only, and more work is needed before general conclusions can be drawn.

(3) *Vitamin B₁ in bread in relation to human requirements.*

Two lines of investigation have given some indication of the vitamin B₁ requirements of man. From his studies of the vitamin requirements of various species of animals Cowgill [1934] deduced that the needs of any species depended on the maximum body weight attained by the species, and the needs of any individual on its particular weight and its calorie intake. He expressed his results in a general formula. From this formula it can be calculated that a man of 70 kg. consuming 3000 calories a day will require about 300 I.U. of vitamin B₁ daily.

The second and more commonly employed method of determining vitamin requirement involves an analysis of the various diets of people who have suffered

from established deficiency diseases, and of those in slightly better conditions who did not develop the disease. Diets associated with the occurrence of beriberi and those giving protection among similar populations have been thus analysed. The difficulty of this method is that there are so few accurate estimations of the vitamins in foodstuffs, by which to evaluate the various diets. In fact it is surprising that with the very inadequate data available concordant results have been obtained by different investigators. Cowgill [1934] investigated a number of diets recorded by other workers and obtained from them data which were in agreement with his formula. Baker & Wright [1936] calculated the vitamin B₁ values of a number of suboptimum and adequate diets, and concluded that beriberi had occurred with those containing 71–382 I.U. daily, and could probably occur with anything under 500 I.U. daily. Stepp *et al.* [1936] considered that 560–1100 I.U. are needed. This last figure is higher than the others.

Two different values for the vitamin B₁ in fresh white bread were found by us, 0.17 and 0.25 I.U. per g., and a value of 0.75 I.U. per g. for wholemeal bread. Therefore, 1 lb. of the white breads contained 77 and 114 I.U. of vitamin B₁, respectively, and 1 lb. of the wholemeal bread contained 341 I.U. If 1 lb. be taken as the amount of bread that may be eaten daily in a rather poor and restricted diet, and if 500 I.U. be taken as the minimum amount of vitamin B₁ necessary for man, it appears that white bread may supply about 1/5 of the vitamin requirements and wholemeal about 2/3. If, on the other hand, the vitamin requirements are only 300 I.U. daily, 1 lb. of white bread will supply about 1/3 and 1 lb. of wholemeal the total amount.

In spite of the relatively low concentration of vitamin B₁ in bread as compared with some other foodstuffs, the large amounts that are commonly consumed may provide a very significant part of the total needs for this vitamin, and indeed in the case of wholemeal bread may satisfy the total needs.

The results obtained with white bread are significant and contradict the general conclusion that all white breads contain very little vitamin B₁. The discrepancy is probably due to the fact that there are considerable variations in the vitamin B₁ contents of different grades of white flour. The white breads tested by us and found to contain enough vitamin B₁ to supply a considerable proportion of human requirements are, we are assured, representative of bread generally used in England. If there were districts in which the bread was made from short extraction patent flour our findings would not apply. In Newfoundland, where beriberi has been observed among white bread eaters on a restricted diet [Aykroyd, 1930], it seems probable that until recently a very highly milled flour was imported for making white bread (private communication from Dr Kent-Jones) and it is likely that where flour is required for making bread in remote districts the tendency would be to use a flour as highly milled as possible to ensure better keeping properties.

Other investigators have already considered the possibility that white bread can supply significant amounts of vitamin B₁. Krogh [1931], while considering that the white wheaten bread eaten in Denmark was an inadequate source of this vitamin, suggested that if the proportion of yeast in the bread was increased five times 1½ lb. daily of bread would be adequate. Since in our experiments with the white breads containing the most yeast only 1/13 of the vitamin in the bread was supplied by the yeast, such an increase would only improve the vitamin content by 1/4. This would scarcely seem adequate.

A number of other investigators have considered the question of improving the nutritive value of bread by adding more yeast, but the maximum amount

used by us, 1 lb. per 100 lb. of flour, is, we are assured by Dr Kent-Jones [see also Eyre, 1933], the largest quantity of active yeast which is commonly employed, and more than this, unless special precautions are taken, will interfere with fermentation. Dried, inactive yeast can be added, but this produces a special bread and is no different from adding any other vitamin-containing material. Scheunert [1936] calculated that 280 g. of a wholemeal bread or 600 g. of a white bread, made with a flour of 75 % extraction, would supply the vitamin B requirements of a man reckoned at a minimum of 300 I.U. a day, but in a later paper [Scheunert & Schieblich, 1937], where the vitamin values for the various flours and breads were not found to be so high and a higher minimum requirement was adopted, it was concluded that not even a wholemeal bread could supply all the vitamin B₁ needed.

There are at present no data available for calculating human requirements of vitamin B₂ complex. Since the activity of wheat flours, in so far as the growth of rats is concerned, is limited by their relative deficiencies of the flavin component of the complex, it is possible that a deficiency of flavin may be the most serious nutritional fault of wheat products.

SUMMARY

1. Estimations have been made of the separate members of the vitamin B complex in various yeasts and flours and in breads made from them. Rat growth methods were employed in testing for each vitamin.

In the tests for vitamin B₁ the international standard acid clay adsorbate was included for reference, and in tests for vitamin B₂ complex and its components baker's yeast (sample III) was adopted as a standard. Determinations were made of the amounts of the test materials promoting weight increases equal to those of 1 I.U. of vitamin B₁ and to those of various doses of the arbitrary standard in the case of vitamin B₂ complex and its components. The results are summarized in Tables IV, IX and XIV.

2. The brewer's yeast was richer in vitamin B₁ than the baker's yeasts, except baker's yeast II which was specially fortified in this respect. All the yeasts were equally good sources of the vitamin B₂ factors other than flavin, and the variations in their flavin contents were not large. The flavin content was the limiting factor for growth in two of the baker's yeasts, including the one from which the breads were made. The vitamin B₂ factors other than flavin were limiting in the brewer's yeast and one baker's yeast.

3. The straight run unbleached and bleached white flours were equal and fair sources of vitamin B₁ and there appeared to be no loss of activity after 12 months' storage. These flours were representative of the ordinary flours commercially used in this country for making bread. A sample of patent flour was found to contain no appreciable amounts of vitamin B₁. Wholemeal flour was about three times as rich in vitamin B₁ as the straight run white flours.

Straight run white flour and wholemeal flour were not found to differ very greatly in their contents of flavin or of vitamin B₂ factors other than flavin; the wholemeal contained rather more of each constituent. The flours were all limited in their growth-promoting activity by their flavin contents which were decreased by bleaching and keeping, while the other B-vitamins were not affected.

4. The vitamin B₁ contents of white breads made by the short or long dough processes were equal and about one-third as great as that of wholemeal bread. The white bread made with baking powder contained no demonstrable

vitamin B₁. The wholemeal bread was a rather better source of vitamin B₂ complex and its constituents than white bread but the difference was not so marked as in the case of vitamin B₁. Flavin was in every case the limiting factor for growth when the breads were fed as source of vitamin B₂ complex.

5. The breads, when dry weights were considered, contained substantially the same amounts of each of the B-vitamins as the flours from which they were made, the only exception being the baking powder bread which contained less vitamin B₁ than the flour. The small amounts of yeast used were not sufficient to raise the vitamin contents of the breads appreciably above those of the flours. No evidence was obtained of synthesis or destruction of B-vitamins in the bread-making process with yeast.

6. The importance of the vitamin B₁ content of bread in relation to human requirements is discussed. The content, although low in comparison with that of some other foods, was found to be sufficiently high for white bread to provide a significant part of the requirements for this vitamin, bearing in mind the large amounts of bread often consumed.

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