XV. VITAMIN B CONTENT OF WHITE BREAD.

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It is frequently assumed that white bread is lacking in vitamin B, yet yeast, which is recognised as being a rich source of this vitamin, is used in bread-making and the temperature reached in baking is not such as would destroy the vitamin.

In 1911 Hill and Flack [1911] fed rats on bread and water, and flour and water in order to compare the relative merits of white, standard and wholemeal breads. None of their animals did well and many died. The rats fed on standard bread did better than those on white bread. These observers state that on wholemeal flour and water the rats "can grow, keep in a very fair condition and beget their own kind." A very obvious criticism of this work in the light of modern knowledge is that the diets used were deficient in fat, vitamin A and mineral salts. Hawk, Smith and Bergeim [1921] found that white bread supplemented with butter and inorganic salts was a diet on which rats could live and put on weight. Their experiments lasted 12 weeks. They used two kinds of bread, one of which was made to approximate closely to home-made bread, and the other contained a large excess of yeast (six times as much). Such bread cannot compare with ordinary bread obtained in this country, because it was mixed with half milk and half water and contained "Crisco," thus making the bread into a more complete food.

The rats fed on the extra yeast bread showed a much better growth and were far superior in general physical appearance. Males were used exclusively in this work and therefore there are no comments as to the possibility of reproduction on this diet.

Eddie and Simpson [1911], working on pigeons, found that standard bread is far superior to white bread, both for adult and young animals. Many of their birds fed on white bread died and showed peripheral neuritis. The condition, however, could be cured by adding yeast to the diet.

Chick and Hume [1917] state that white flour is deficient in the anti-neuritic vitamin, and if used as an exclusive diet for pigeons will produce polyneuritis. It is, however, possible that some animals are more susceptible than others to lack of vitamin B.

As the result of these experiments it was natural to assume that baker's ordinary white bread lacks vitamin B. Critically examined the work of Hill and Flack cannot be accepted as a criterion, since their diets were deficient in other respects, and the experiments of the American workers throw no light on the subject because they use whole milk in the making of the bread, and this food has itself been shown to contain vitamin B, while those of Chick and Hume concern the vitamin B content of flour, not that of baker's bread which has a considerable admixture of yeast, and so may be expected to contain vitamin B. Consequently it was thought necessary to make experiments on young growing rats giving white bread as the sole source of vitamin B.

METHODS.

(a) *Animals.* Rats alone were used in the experiments to be described in this paper. The animals were all bred in the laboratory and their mothers had good mixed diets, both during pregnancy and lactation.

It is usual in most laboratories to take rats of 40 g. weight as the maximal size when the experiments start. With the ordinary stock bought from an animal dealer this corresponds to an age of between 30 and 40 days [Donaldson, 1906]. But with the stock used, bred along lines described by Hartwell, Mottram and Mottram [1923], the mean weight of 40 g. corresponds to an age of 21 days, *i.e.* the day upon which we are accustomed to wean them, whereas animals 38–39 days old weigh no less than 70 g. Consequently two sets of experiments have been made,

- (1) with animals of the average *weight*; and
- (2) with animals of the average *age*, used in other laboratories.

When the special diet was not started until the animals weighed approximately 70 g., the rats were fed, as are the stock animals, on a diet of kitchen scraps and a small amount of bread and milk.

(b) *Foods.* The diet consisted of bread, butter and salt mixture with sufficient water to enable the constituents to be well mixed. The proportions were kept constant, *i.e.* 30 g. bread, 3 g. butter, 0.7 g. salt mixture [Hartwell, 1922, 1], 60 cc. water, and the animals were fed so that small amounts were left—*i.e.* they had as much as they would eat. The bread was weighed one day old. Two kinds of bread were used:

1. White bread bought from a baker.
2. Home-made bread.

This bread was made for me by Miss M. D. Hamilton-King, in the Household Work Dept. of this college, and two varieties were available, *viz.* baking powder bread and yeast bread. The composition of both is given below. White flour (Household) was used in the preparation of both:

Baking powder bread	Yeast bread
3½ lbs. flour	3½ lbs. flour
8 g. salt	8 g. salt
15 g. sodium bicarbonate	20 g. sugar
25 g. cream of tartar	30 g. yeast

(c) *Technique*. For the majority of the experiments 12 animals were selected (six of each sex) and kept in a cage together. They were weighed separately each day, except Saturday and Sunday. They had moist food, but no water to drink.

EXPERIMENTS.

I. *Baker's bread*.

(a) *Preliminary experiments*.

A. Two male rats fed on bread, butter and salt mixture and water for *one year*. Weight at beginning of experiment 96 g. and 115 g. Weight at end of experiment 325 g. and 374 g.

B. Two male rats fed on bread and butter and water for 8½ months. Initial weights 101 g. and 106 g.; weights increased to 246 g. and 232 g. respectively.

(b) *Further experiments*. Diet: bread, butter, salt mixture and water.

A. Animals started at approximately 40 g.

Exp. 1. 8 rats used, 4 ♀ and 4 ♂. Feeding continued 7½ months.

„ 2. 12 rats used, 6 ♀ and 6 ♂. „ 5 „

B. Animals started at approximately 70 g.

11 rats used, 6 ♀ and 5 ♂. *Exp.* lasted 4½ months.

II. *Baking powder bread—home-made*.

A. Animals started at 40 g.

12 rats used, 6 ♀ and 6 ♂. *Exp.* lasted 12 weeks.

B. Animals started at 70 g.

12 rats used, 6 ♀ and 6 ♂. *Exp.* lasted 6 weeks.

III. *Yeast bread—home-made*.

A. Animals started at approximately 40 g.

12 rats used, 6 ♀ and 6 ♂. *Exp.* lasted 12 weeks.

B. Animals started at approximately 70 g.

10 rats used, 6 ♀ and 4 ♂. *Exp.* lasted 11 weeks.

Results. The rats were more susceptible to cold than are normal animals. After 2–3 weeks on the diet, the fur came out in patches; practically all the fur came off, but eventually a new coat grew, and this was quite equal to that of the normal rat. Other work now in progress, suggests that this is due to lack in quality (and possibly also quantity) of the protein.

The growth was slow, but fairly regular. The males did less well than the females, and grew more slowly, the reverse of what occurs in normal animals.

The does produced litters; the babies were of low weight and frequently one or more were born dead. Some of the young were reared. (Details of this will be published later.)

The rats fed on baking powder bread did not thrive as well as the others, and in every case the animals put on to the diet at 70 g. had a distinct advantage, *e.g.* their coats did not come out so badly, etc.

IV. *Growth of rats on bread and water.*

As a further test of the vitamin B content of white bread, it was decided to try to keep some animals on bread and water alone. Males were used exclusively because previous experiments have shown that they do less well than the females on a poor diet. It has been shown by various observers that lack of vitamin B is evidenced before any other dietetic disturbances, hence if vitamin B were absent it would be noticed before the effects of any other deficiencies became evident.

The results of the experiment are tabulated below, and offer good evidence for stating that there is an adequate supply of vitamin B in white bread as bought from a baker.

Length of exp. in days	Weight of rat in g.		Gain in g.
	Beginning	End	
27	259	345	86
27	281	315	34
21	302	315	13
21	320	368	48
21	45	59	14
21	47	58	11
21	45	68	23
21	45	55	10
21	44	68	24
21	47	68	21

From these experiments it can be seen that there is sufficient vitamin B in white bread to provide for the needs of the rat. The growth, however, was by no means good and hence it might be suggested that there is a small amount only of vitamin B in such a diet and that if more were added the growth would improve. To test this the following experiment was made.

V. *Growth of rats on white bread, butter, salt mixture, and wheat germ extract.*

The proportions of the bread, butter, and salt mixture were the same as before, but wheat germ extract was used instead of water. The wheat germ¹ was placed in a Winchester quart bottle with warm distilled water (6 g. germ to 100 cc. water) and shaken on a mechanical shaker for 1½ hours. The supernatant liquid was then decanted, and the amount measured. This was boiled to coagulate some of the protein, cooled and the volume made up with distilled water to what it was before boiling. This liquid was filtered and the filtrate used to soak the bread, *e.g.* 60 cc. to 30 g. bread.

This experiment lasted 10 weeks. The condition of the rats was not at all improved by the addition of extra vitamin B. They were just as susceptible to cold and their fur came off in exactly the same way. Several animals were practically bald on their chests and abdomens. The fur grew again, as it did on the other rats fed with bread, butter, salt mixture and water.

¹ I wish to thank Mr C. Thompson of Penhill Close, Cardiff, for providing me with wheat germ.

The growth of the females was practically the same as before, but the males did slightly better. The improvement was *very* slight, and it is possible that it could be explained as being due to the small amount of extra protein added with the wheat germ extract.

VI. *The vitamin B content of wholemeal and white flours.*

It has been shown previously [Hartwell, 1922, 2] that an excess of protein in the lactating rats' diet is detrimental to the sucklings, unless a large proportion of vitamin B is also included. This fact can be utilised to test the vitamin B content of a food, and the following experiments were made in order to see if white and wholemeal flours contained any vitamin B.

Two samples of flour¹ were available. Sample A, wholemeal, was ground on stones, and represents 100 % of the wheat. Sample B, white flour, was made from exactly the same wheat as A, but was to an extraction from clean wheat of about 72.5 %.

Preparation of flour extracts. The flour was mixed to a smooth paste with a small quantity of distilled water, and this paste washed into a Winchester quart bottle. The proportion of flour to water was 12 g. to 100 cc. This mixture was shaken on a mechanical shaker for 1½ hours, and the subsequent preparation was the same as that described on p. 123 for the preparation of wheat germ extract.

Experimental. The mother rats were fed an excess protein diet, one which had been found to produce bad symptoms in the sucklings, and the flour extract was added to this diet. The method adopted was that described in a previous paper [Hartwell, 1922, 1]. Six mother rats were given the excess protein diet, with the addition of extract of A flour, and six more had a similar diet with extract of B flour added.

The babies whose mothers were fed with A extract (from wholemeal flour) did better than those whose mothers had B extract, *i.e.* there is more vitamin B in wholemeal flour than in white flour. This is a very obvious statement, for it is well known that wholemeal flour contains much more germ than white flour, but the point of interest is that two of the six litters whose mothers had B extract survived, whereas such a diet, without the addition of vitamin B, has previously resulted in the death of all litters. These results, therefore, are in agreement with those described earlier in this paper, in which rats grew and showed no symptoms of lack of vitamin B on a diet of baking powder bread, butter and salt mixture.

VII. *Comments.*

i. *Criticism of experimental procedure.*

In most of this work, the rats were kept in metal cages and had access to the sawdust in the tray at the bottom. Steenbock, Sell and Nelson [1923] have shown that rats, kept on a low vitamin B diet are apt to eat their excreta

¹ I have to thank Mr J. McDougall for sending me the flour.

and hence obtain a further supply of the vitamin. This work was not published when the experiments described in this paper were started, otherwise special precautions would have been taken in this respect. However, an extra experiment (not quoted in the text) has been made in which 12 rats (six female and six male) were kept in a cage with wire meshwork floor, and the tray well below the level of the floor of the cage. The sawdust was changed daily, and therefore the rats had no chance of eating excreta. The rats were fed on baker's bread, butter, salt mixture and water, as before, and the growth curves were identical with those quoted in this paper. The experiment lasted 10 weeks.

Also in experiments I, i and ii, and IV, the animals were kept in similar cages.

It is possible that the butter may contain a trace of vitamin B in the butter-milk. In the 10-week experiment referred to above, the animals were fed on "washed butter" (i.e. butter stirred with hot water until melted and the fat when cold allowed to drain through muslin and then pressed as free as possible from liquid).

ii. *Comparison with work on similar lines by other workers.*

The experiments of Hawk, Smith and Bergeim are quite parallel with those described in this paper, except that their bread had a higher nutritive value in that a mixture of milk and water was used in the making. In spite of this, a rather better growth curve is given in the author's experiments, which on the whole agree with those of the American workers.

iii. *Explanation of slow rate of growth.*

It is suggested that the slow rate of growth is due to the lack in quality and quantity of protein. Hawk, Smith and Bergeim [1921] obtained far better growth with "extra yeast" bread, which would certainly contain more protein, since yeast contains 11.7 % of protein. The essential amino acids are poorly represented in wheat protein, and experiments now in progress show far better growth when a good protein is added to the diet of bread, butter, salt mixture and water.

iv. *Comparative growth of male and female.*

As a rule the male animal grows more rapidly than the female. It is extraordinary, that, in these experiments the rate of growth of the two sexes is practically equal, if anything the females are the heavier (see composite curves, Fig. 1). When wheat germ extract was added, the males did rather better than the females. Therefore it appears that, on a poor diet, the female can either make a more economical use of what foodstuffs are available, or that she is accustomed to sacrificing herself for the good of her offspring and is therefore less affected than the male by a poor diet.

The individual curves show that the males have not such good growth as the females, especially when the diet is started at an early age.

Fig. 1. Composite curves (6 ♂ and 6 ♀).

1. Control animals, fed on bread and milk and kitchen scraps.
2. 30 g. baker's bread, 3 g. butter, 0.7 g. salt mixture, 60 cc. wheat germ extract.
3. 30 g. yeast bread (home-made), 3 g. butter, 0.7 g. salt mixture, 60 cc. water.
4. 30 g. baker's bread, 3 g. butter, 0.7 g. salt mixture, 60 cc. water.
5. 30 g. baking powder bread (home-made), 3 g. butter, 0.7 g. salt mixture, 60 cc. water.

VIII. Conclusions.

White bread contains sufficient vitamin B to supply the needs of a rat, both for growth and reproduction. On a diet of white bread, butter, salt mixture and water, the growth is slow, but continuous. This slow growth is probably due to deficiency in the quality and quantity of the protein.

White flour contains a little vitamin B, but the main source is the yeast.

I wish to thank Miss Margaret Dyer for putting her knowledge of types of flour at my disposal and for interesting Mr J. McDougall in the work, Miss M. D. Hamilton-King for making the home-made bread used, and Prof. V. H. Mottram for his unfailing criticism and help.

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