CVI. VITAMINS IN HUMAN NUTRITION

VITAMIN B₁, AND THE "BROWN VERSUS WHITE BREAD PROBLEM". I

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IN a report issued by the Ministry of Health, Lowry & Pearse [1929] stated that "the diet in the households of the unemployed men [in the South Wales coalfields] comprised little beyond white bread, butter or margarine, potatoes, sugar, jam, tea and bacon in limited quantity", and expressed the opinion that "such a diet could be much improved if greater use were made of wholemeal bread". The point here raised is one of much interest in human dietetics, and it was thought worth while to undertake some animal feeding experiments to examine more fully various aspects of the question. At the outset our principal objects were (1) to compare the vitamin B₁ contents of several diets of this kind, as actually taken in the mining area, and (2) to explore the possibility of improving them by addition of wholemeal bread or in other ways.

Refecation as an experimental difficulty. In our earlier tests carried out in 1930 and 1931 we estimated vitamin B₁ by the standard growth-rate method with rats. Much to our surprise, little difference was found in the growth responses as between white bread, wholemeal or "germ bread". This result recalled to mind some earlier findings of Hartwell & V. H. Mottram [Hartwell, 1924; Hartwell & V. H. Mottram, 1929], which seemed to show that white bread might sometimes be a satisfactory source of vitamin B—a conclusion questioned by Cramer & J. C. Mottram [1927] and others (for further consideration of this point see discussion below). We soon found, however, that the explanation was that our rats had become "refected", as a consequence apparently of the inclusion of the bread in their diet. Refecation has been described by a number of past workers [Fridericia, 1927; Roscoe, 1927; 1931; S. K. Kon & Watchorn, 1927; Mendel & Vickery, 1929; S. K. Kon, 1933] as brought about by the use of raw potato starch (or occasionally on other prepared starches), but there seems no previous record of its having been seen with bread.

The conditions under which refecation may occur appear to vary considerably from one laboratory to another. In some institutes the phenomenon has rarely or never been seen; in our own the use of diets rich in various starchy foods (not necessarily potato starch or wheat starch) may frequently suffice to procure refecation in the majority of animals. Presumably an animal-house may become infected with the refecation-producing organisms, after which refecation will readily occur. Specially prepared "vitamin-free rice starch", as used in a number of our basal diets, in contrast with certain naturally occurring foodstuffs rich in starch, has not generally in our experience been a cause of refecation. But even with rice starch occasional instances of refecation have been seen. Potato starch almost always brings about refecation, in this laboratory. The difficulty of establishing a definite criterion for refecation, at least in its earlier stages, is referred to below. [For fuller literature on refecation see P. M. Kon, 1935.]

Alternative methods for determination of vitamin B₁. Since the growth tests with rats were thus rendered nugatory by the occurrence of refecation, it became

necessary to seek other methods for estimating vitamin B₁ in bread. A trial was made next of a method based on cure of convulsions in rats [Smith, 1930; see also Birch & Harris, 1934] but this led again to unsatisfactory results (p. 804).

Advantages of the bradycardia method. Finally recourse was had to the so-called bradycardia method [Birch & Harris, 1934; see also Harris, 1934; Leong & Harris, 1937, 1; Baker & Wright, 1935], which has been evolved out of the observations of Drury & Harris [1930]. This was found to give satisfactory results. It possesses two special advantages for work of this kind. (1) A single dose only of the food to be examined is given and the subsequent experimental period is a very short one and hence there is little chance of refection supervening. (2) The animal is used at a time when it is still free from any advanced symptoms of deficiency, so that its appetite and powers of absorption remain virtually unimpaired: hence when the substance under test is only a poor source of the vitamin large amounts of it can be ingested. In fact it would appear that the bradycardia method is the only satisfactory technique available when the foods to be assayed are of a highly starchy nature and contain but small traces of vitamin B₁.

Scope of present enquiry. The work to be described eventually resolved itself into a study of three principal questions. (1) A comparison of wholemeal bread with "germ bread". (2) A comparison of "bran bread" (=germ-free wholemeal bread, made by admixture of white flour with bran) with genuine wholemeal bread and with white bread. (This second problem was undertaken at the request of the former Government Chemist, Sir Robert Robertson, by whom it was pointed out that little work had hitherto been published on the comparison of genuine wholemeal with other brown loaves and of the latter with white loaves.) (3) A comparison of the corresponding flours. (This last aspect is dealt with principally in the following paper [Leong & Harris, 1937, 2].)

EXPERIMENTAL

I. PRELIMINARY EXPERIMENTS

Occurrence of refection on bread diets

Full reference has already been made above to the occurrence of refection in growth tests with bread. As no earlier workers appear to have had the experience that refection may be caused by the use of bread, it seems advisable to give an outline of the experimental conditions.

Exp. 1. Attempted comparison of white bread and "germ bread" by growth tests. A large number of rats were first depleted of the vitamin B complex, upon the usual basal diet used in this laboratory, the carbohydrate being provided for this experiment in the form of rice starch. When the rats had begun to lose weight, half of them were placed upon the following diet:

White bread ...	...	...	...	100
Skimmed sweetened condensed milk	...	...	...	7
Margarine ("Fresh Roll")	...	...	...	18

and the remainder on the same diet with "germ bread" substituted for white.¹ All the rats started to gain weight almost immediately, and little difference was to be seen between the two groups (Fig. 1).

¹ Some animals in this and subsequent tests received cod liver oil daily in addition, but little or no difference was seen in their behaviour.

Exp. 2. In another test carried out at the same time, two further groups of rats after depletion on the same basal diet were fed on diets consisting solely of (1) white bread and (2) "germ bread". As in *Exp. 1*, a resumption of growth occurred almost immediately the bread diet had been started, and there was little difference between the "germ bread" animals and the white bread animals (*Fig. 2*).

Fig. 1 (above) and *Fig. 2* (below). Growth curves of rats on "germ bread" and white bread. — "Germ bread". ---- White bread.

Absence of criteria of refection. In the earlier stages of these two experiments, while the animals were already gaining weight steadily on the diets of white bread or "germ bread", little departure from normality could yet be seen in the appearance of the faeces. Later on, the faeces became characteristically bulky and grey. We have repeatedly had the same experience in other experiments in which refection has been induced, e.g. with potato starch. Refection seems insidious in its onset, and it is often difficult to diagnose it from the appearance of the faeces until quite a late stage in its development. While the peculiar bulky "white" faeces can no doubt be held to be a criterion of refection, their absence cannot be taken to imply that refection has not started. This circumstance makes the diagnosis of refection a matter of some difficulty, as well as a cause of much possible confusion.

At the conclusion of *Exps. 1* and *2* the rats were returned to a vitamin B-free basal diet containing the carbohydrate in the form of rice starch. They thereupon promptly lost weight, and died with typical "polyneuritic" symptoms.

Exp. 3. Comparison of various starches. A third experiment was conducted along similar lines (July 1930). After the usual depletion period, diets consisting solely of "germ bread" or white bread were given. As in *Exps. 1* and *2* both groups thrived equally well (*Fig. 3*), and after 95 days all the animals were transferred to a basal diet free of vitamin B but containing carbohydrate in the form of potato starch. Most of the animals continued to show further slight gains in weight and the faeces were observed to be pale and bulky. After a further 40 days the potato starch in the diet of two of the animals was replaced by

purified rice starch. A rapid loss in weight promptly occurred. Growth was resumed when a source of vitamin B (marmite) was added to this diet, and later, indifferent growth followed afterwards by a rather slow decline was observed when a vitamin B-free diet containing potato starch in place of rice starch was once again employed (Fig. 3).

Fig. 3. ——— "Germ bread". ——— White bread. Basal diet. ---- Basal diet + potato starch. —•— Basal diet + rice diet. — Basal diet + marmite.

It would seem then that the process of refection could continue when the diet contained potato starch, but that rice starch was generally not an effective substrate for refection. (As already mentioned an occasional animal may become relected on rice starch.)

Rat-growth tests with white flour and "germ flour"

In the next experiments rats were tested with white flour and with a proprietary "germ flour", in place of the corresponding breads. In contrast to the results with the breads, a distinct difference in growth rate could now be seen, better growth resulting with the "germ flour" than with white flour. It appeared that flour was less efficient than bread as a substrate for refection. Perhaps the main interest of this result is the contrast with observations on cooked and raw starches. Several workers have found that refection occurs readily on raw potato starch but not on cooked potato starch.

Experimental details of these tests follow:

Exps. 4 and 5. In Exp. 4 (carried out in October 1930) rats were first depleted of vitamin B on the usual basal diet with purified rice starch as the source of carbohydrate and then placed in equal groups on diets of white flour and "germ flour". A slow rate of growth resulted with the white flour, but a much better growth with the "germ flour" (Fig. 4). Animals on white bread given additional vitamin B, as marmite, made improved gains (not shown in the figure). Negative controls duly lost weight and died.

Fig. 4. Growth curves of rats on white flour and "germ flour" following basal diet of starch. — "Germ flour". ---- White flour.

Fig. 5. Growth curves of rats on white flour and "germ flour" after preliminary basal diet of sugar. — "Germ flour". ---- White flour.

In a parallel experiment (Exp. 5, Fig. 5) the preliminary basal diet contained 60% of sucrose in place of rice starch: this change was made with the idea of seeing whether it might delay the incidence of any possible refection on the subsequent flour diets. No difference, however, was seen as compared with Exp. 4. At the conclusion of the experiment the rats were transferred to diets containing potato or rice starch. They generally lost weight slowly on potato starch and more rapidly on rice starch, but the experiment was inconclusive from this point of view.

It will be conceded that it is difficult to say from these trials to what extent the slow gains observed with the white flour were due to some degree of refection having occurred or to the presence of small amounts of vitamin B₁. Subsequent work suggests that it is mainly the latter, but it may be concluded that in this laboratory the rat-growth method has proved unsatisfactory for the assay of farinaceous foods containing small quantities of vitamin B₁ because of this uncertainty of the complicating factor of refection.

Attempts to estimate vitamin B₁ in bread by cure of convulsions in rats

During 1931-2 attempts were made to estimate vitamin B₁ in various types of bread by means of the method of cure of convulsions in rats. As the method was not in the end considered suitable for this purpose, no detailed account of the results need be given here. It is sufficient to say that our general finding was that the rat when already suffering from advanced polyneuritis seems to have difficulty in absorbing the relatively large amounts of bread that have to be fed. We endeavoured to overcome this difficulty by giving a substance such as marmite (which effects a rapid cure) simultaneously with the bread to be tested, in the hope of observing a summative effect and so being able to assess the response due to the bread by difference. This expedient, however, was not found satisfactory. It should nevertheless be emphasized that for the estimation of vitamin B₁ in more active sources, such as wheat germ, yeast, standard acid clay, and various concentrates, etc. the "rat-convulsions" method was found to answer admirably, as has been reported elsewhere [Birch & Harris, 1934].

II. COMPARISON OF VARIOUS BREADS BY THE BRADYCARDIA METHOD

Exp. 6. Preliminary comparison of white bread and "germ bread". For the purpose of a preliminary trial of the bradycardia method as applied to breads, determinations were first made on ordinary white bread and on a proprietary "germ bread". As we desired at this stage to obtain round figures merely of the approximate orders of potencies of the two types of bread, this experiment was carried out with a limited number of animals only. The technique was that described in papers previously published [Birch & Harris, 1934; Harris, 1934]. The loaves of bread were obtained from local retailers. The responses observed are set out in Table I; and the corresponding dose-response curves have been combined into a single curve for all three substances, by suitable adjustments of

Table I. *Preliminary comparison of white bread, "germ bread" and international standard (1934)*

Material	Dose	Duration of cure (days)*	
		Individual animals	Mean
International standard (activated acid clay)	20 mg.	5, 6.5	5.75
	30 "	6.5, 8.5, 9.5	8
	40 "	7, 9, 11.5	9
"Germ bread"	1 g.	2.5, 2.5, 2.5, 3.5	2.75
	2 "	4, 4.5, 5, 5.5	4.75
	4 "	5.5, 6, 8, 11.5	7.75
White bread	2 g.	3, 3.5	3.25
	4 "	2.5, 3.5	3
	8 "	2.5, 4, 6	4.25

* Intermediate cures, e.g. greater than 6 days but less than 7, are uniformly reckoned as half-days, i.e. 6.5 days.

the scales of abscissae, in Fig. 6. The corresponding activities, in terms of I.U. per g. of bread, are entered in the final summary of results (Table IV). It will be observed that these preliminary findings have been confirmed in later more precise determinations (Table IV).

Fig. 6. Dose-response curve with international standard, "germ bread" and white bread.

X I.S. ● "Germ bread". ⊙ White bread.

Exp. 7. Comparison of "with germ" and "without germ" bread. Through the initiative of Sir Robert Robertson, to whom I wish to express my indebtedness, specimens of bread were baked under identical standardized conditions at the experimental bakery attached to the Supply Reserve Depot, Woolwich, under the supervision of Messrs J. King and J. A. Heald. The first test to be made involved a comparison of loaves prepared from wheaten flours, (a) wholemeal with the germ included and (b) wholemeal from which the germ had been abstracted (described as "with germ" and "without germ" respectively). The purpose was to ascertain whether "brown bread" containing the branny matter, but without the germ, was greatly inferior to genuine wholemeal bread, containing the germ. The results (Tables II and IV, Fig. 7) indicate that the variety of "with germ" bread examined was not markedly superior to the "without germ" bread. Further tests carried out in greater detail upon various "bran breads" or "with germ" and "without germ" breads, to be recorded below, confirm this result (see Table III).

Table II. Comparison of "with germ" and "without germ" breads (May 1934)

Material	Dose	Duration of cure (days)	
		Individual animals	Mean
International standard (activated acid clay)	20 mg.	3, 3, 3.5, 5.5	3.75
	30 "	3.5, 4.5, 5, 7.5	5
	40 "	5, 8, 10	7.5
"With germ" bread	2 g.	3, 3, 3.5	3
	4 "	5, 7, 7.5, 7.5	6.75
	6 "	5, 7, 7.5, 7.5	6.75
"Without germ" bread	2 g.	2.5, 2.5, 2.5, 3.5	2.75
	4 "	6, 6.5, 6.5, 7	6.5
	6 "	6.5, 7.5, 8.5	7.5

Fig. 7. Dose-response curve with international standard, "with germ" bread and "without germ" bread.

× International standard. ■ "With germ" bread. □ "Without germ" bread.

Flattening of dose-response curve. An incidental finding (cf. also Fig. 2) is that as larger doses of bread, e.g. 6 g., are given there is sometimes a flattening of the dose-response curve, as though the vitamin is then less completely absorbed than when smaller amounts are given. A similar phenomenon has been observed by other workers in the course of vitamin B₁ assays. It seems preferable under these circumstances to restrict the comparison to "medium" intakes of the unknown only, whenever possible—i.e. to doses which give a response in the range corresponding with about 10–30 mg. of international standard acid clay. With the latter material, as with concentrated preparations of vitamin B₁ in general, the curve remains linear up to a high level of

dosage (i.e. when expressed in terms of units of vitamin B₁), probably because absorption and utilization are more complete than when bulky foodstuffs containing a considerable amount of undigested residue are used.

Table III. *Comparison of various kinds of bread from the Government Laboratory, December 1934*

Material	Dose	Duration of cure (days)	
		Individual animals	Mean
International standard (activated acid clay)	10 mg.	3, 3, 3, 4	3.3
	20 „	4, 5, 6, 7	5.5
	30 „	7, 8, 10	8.3
White bread	4 g.	2, 2	2.0
	6 „	3, 3	3.0
	8 „	3, 4	3.5
"With germ" bread	1 g.	3, 3, 4, 6	4.0
	2 „	4, 5, 5, 6, 6, 6	5.3
	4 „	6, 6, 7, 8, 8	7.0
"Wholemeal" bread	1 g.	3, 4, 8	5.0
	2 „	4, 8	6.0
	4 „	6, 7	6.5
"Without germ" bread	1 g.	3, 3, 4, 4, 4, 5	3.8
	2 „	3, 3, 3, 5, 6	4.0
	4 „	7, 7, 7, 9	7.4
"Bran" bread	1 g.	2, 2, 3	2.3
	2 „	2, 2, 3, 4	2.8
	4 „	6, 6	6.0

Exp. 8. Comparison of white, "with germ" and "without germ", and "wholemeal" and "bran" breads. These specimens of bread were again obtained from the Government Laboratory by arrangement with Sir Robert Robertson. The object was to confirm the preliminary finding that brown bread containing little germ may still be a good source of vitamin B₁, and in this respect preferable to white bread. The following descriptions of the various types of bread are kindly provided by Sir Robert Robertson and Mr More of the Government Laboratory.

(a) "*With germ.*" The meal was obtained by grinding the whole wheat grain without separation of germ or bran, i.e. it is a 100% wholemeal.

(b) "*Without germ.*" The meal is similar to the above except that the germ was removed as "germ offal" as completely as is commercially possible, the bran content being the same as in (a).

(c) "*Wholemeal.*" This is a wholemeal as supplied to the Army Supply Depot, and not specially prepared for these experiments. Germ and bran are present.

(d) "*Bran.*" These loaves were made from a meal prepared by mixing in the laboratory white flour with finely ground bran, in the proportions in which they occur in wholemeal. The meal so prepared would be practically free from germ and correspond therefore with the "without germ" meal.

(e) "*White.*" Prepared from unbleached white flour.

The meals (a) and (b) were specially milled by Messrs Cranfield Brothers, Ipswich, from the same wheat mixture.

The details of the baking process were as follows:

	Weight taken
Meal or flour	23.5 oz.
Salt	8 g.
Yeast ("D.C.L." compressed yeast)	12 g.
Water	425 ml.
Time of "proving" from addition of yeast to oven ...	2.5 hr.
Ditto, for white flour	2.75 hr.

Fig. 8. Dose-response curve with various kinds of breads, baked in Government Laboratory.
x International standard. ♦ Wholemeal bread. ■ “With germ” bread.
□ “Without germ” bread. ◇ Bran bread. ○ White bread.

Table IV. *Vitamin B₁ values of breads. Collected results*

	Activities, i.u. per g.			
	Preliminary trial (1) (1934). Breads purchased locally	Preliminary trial (2) (1934). Breads baked in Government Laboratory	Main test (1935). Breads baked in Government Laboratory	Confirmatory test (1935) [Leong & Harris, 1937, 2]. Breads purchased locally
White bread	0.15	—	0.12	—
Proprietary “germ bread”	0.8	—	—	0.9
Proprietary “wholemeal” bread	—	—	—	0.75
Specimens from experimental bakery:				
“With germ” bread	—	0.8	0.9	—
“Wholemeal” bread	—	—	0.9	—
“Without germ” bread	—	0.8	0.65	—
“Bran” bread	—	—	0.5	—

The results of this test are recorded in Table III, and the composite dose-response curves are shown in Fig. 8. The principal finding (see Table IV) is that "without germ" bread or "bran" bread is a reasonably good source of vitamin B₁ not greatly inferior to genuine wholemeal bread or "with germ" bread.

The flattening of the dose-response curve noted for Fig. 7 is again seen in Fig. 8.

For the sake of comparison Table IV also gives the results of a later confirmatory test carried out with Mr P. C. Leong in 1935 [see Leong & Harris, 1937, 2], in which the activity of "germ bread" was again determined, together with that of a proprietary brand of wholemeal bread. The values found are in keeping with the earlier conclusions.

DISCUSSION

It is generally recognized that genuine wholemeal bread is superior to white bread as a source of vitamin B₁, but it has been much questioned of late whether ordinary "brown bread" as commonly sold and containing no appreciable amount of germ is for this reason preferable to white bread. No doubt can remain that this question must now be definitely answered in the affirmative.

Perhaps the most novel of the findings recorded in this paper, however, are (1) the observation that refection may be induced by diets of white or brown breads, and (2) the surprising result that the variety of "germ bread" which we examined was not greatly superior in vitamin B₁ content to the "wholemeal" bread, or the latter to "bran bread".

The matter of refection may be discussed first. As already mentioned Hartwell recorded in 1924 that she had found that "white bread contains sufficient vitamin B to supply the needs of a rat, both for growth and reproduction". She supposed that while the flour contained a small amount of vitamin B the main source was the added yeast. Hartwell & V. H. Mottram [1929] argued from these results that propaganda urging the use of brown bread by the working classes were uncalled for. The conclusions of Hartwell were, however, called into question by Cramer & J. C. Mottram [1927] who reasoned that the amount of yeast ordinarily present in bread was so small as to be of little practical significance as a source of vitamin B. They calculated that the vitamin B value of wholemeal bread corresponds with the amount known to be present in its component parts (including germ, endosperm, pericarp; cf. Table V), and concluded that the good growth of the rats on white bread seen in the rats in Hartwell's experiment must presumably have been due to the butter which was added to their diet. An alternative explanation now seems to offer itself on the basis of our own experiences, namely that the rats in Hartwell's tests had become repleted. Repletion has occurred with such regularity in our experiments, that when the fact is also borne in mind that the signs of it are often not readily noticed, this suggested explanation seems likely enough.

The second point which calls for discussion is the fact that the proprietary "germ bread" examined was not such an active source of vitamin B₁ as might have been expected from the generally accepted high value for the fresh germ itself, and further that bran bread (or "without germ" bread) was not markedly inferior to genuine wholemeal bread (or to "with germ" bread). The values for the various wheat products as collected by Cowgill [1934] from the available literature [see Cowgill for the original references; also Morgan & Frederick, 1935; Morgan & Hunt, 1935] are as follows (Table V).

Table V. "*Relative vitamin B₁ index*" (Cowgill)

Bran	40	Middlings	75
Flour	3	Whole wheat	20
Germ	135	White bread made with water	2

The finding that "without germ" bread or "bran bread" is not greatly inferior to "with germ" or wholemeal may be sufficiently accounted for from the fact that the bran and middlings are a fairly potent source of vitamin B₁ (see Table V). A redetermination of the vitamin B₁ contents of bran and middlings, by means of the bradycardia method, is reported in Part II [Leong & Harris, 1937, 2] and the result leaves no room for doubt that these materials are if anything even more active than has hitherto been recognized. Thus although inferior to the germ, when expressed in terms of units per g., the relatively larger amount of such branny matter present would have a great influence on the final vitamin B₁ value of the bread (see Cramer & J. C. Mottram [1927] for calculations of this kind). The same reason, partly at least, accounts for the fact that the proprietary "germ bread" examined was not found to be greatly more active than wholemeal bread. The "germ bread" in question is baked from a flour prepared by admixture of 3 parts of white flour with 1 part of a prepared wheat germ, and thus, in contrast with the wholemeal bread, has none of the branny matter as a source of vitamin B₁. A further possible explanation would be that there is presumably an appreciable loss of vitamin B₁ during the cooking process to which the germ is first subjected before incorporation in the flour, or again the variety of germ used may not be of the highest vitamin B₁ potency. These points are referred to further in the following paper [Leong & Harris, 1937, 2], where the data for wheat germ are reviewed, and the calculated or "expected" values for "germ flour" are compared with the values actually found.

SUMMARY

Section I. Attempts to compare the vitamin B₁ contents of white and brown breads either by (1) the rat-growth method, or (2) the cure of convulsions in rats, failed to give satisfactory results. With the rat-growth method, refecation supervened, and was found to be an insuperable obstacle because (1) of its insidious onset, (2) of the absence of criteria for its presence (the pale enlarged faeces may not be seen until it has been established for some time—and then sometimes only erratically). Refecation seems to occur much more readily in some laboratories than in others.

The method based on the cure of convulsions in rats was also found unsatisfactory, the principal difficulty being to get the rats when already seriously ill with convulsions to consume the relatively large amounts of bread which would be necessary to provide an adequate amount of vitamin B₁ for a cure. The same difficulty is encountered with the pigeon curative test, and in addition the percentage error of the method is known to be high.

Section II. The bradycardia method overcomes these several difficulties and enables an accurate assay to be carried out. As only one dose of unknown is given there is little chance of refecation becoming established, and as the animal is tested before it is gravely ill and while its appetite is still good, large amounts of the unknown may be satisfactorily administered when they are relatively poor sources of the vitamin.

The results with various types of bread are summarized in Table IV. The main points of interest are: (1) A proprietary "germ bread" (prepared from a

mixture of 3 parts of white flour plus 1 part of treated germ) was found to be but surprisingly little superior to ordinary wholemeal bread, although about 7–8 times more potent than white bread. (2) Specimens of “without germ bread” or “bran bread” (i.e. “brown breads” containing the branny matter but with a minimum of germ) which were examined were superior to white bread in containing significant amounts of vitamin B₁ and, contrary to expectation, were not greatly inferior to genuine wholemeal bread or to “with germ” bread (i.e. breads containing in addition the small proportion of germ present in the original whole wheat).

I am greatly indebted to Sir Robert Robertson for his suggestions and help, to members of his staff at the Government Laboratory for co-operating in the supply of loaves baked under standardized conditions, and to A. Ward for his skilled care of the experimental animals.

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