LV. THE DISTRIBUTION OF VITAMIN B₂ IN CERTAIN FOODS.

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By vitamin B₂ is here meant the factor in the vitamin B complex, other than the antineuritic, which promotes growth and cures and prevents dermatitis in rats. Its possible identity with the "P. P." factor, preventive and curative of human pellagra, and probably of "black-tongue" in dogs, is discussed later in this paper.

Since the discovery that McCollum and Davis's [1915, 1, 2] water-soluble B vitamin can be subdivided into at least two factors, Eijkman's [1897] antineuritic and another [Smith and Hendrick, 1926; Goldberger *et al.*, 1926; Chick and Roscoe, 1927], no attempt has been made to work out systematically the distribution of the more heat-stable factor, vitamin B₂. The distribution of the other constituent, the antineuritic vitamin B₁, as investigated by the power of a food to protect a bird on polished rice from polyneuritis or to cure the condition when it is developed, is presumably not affected by the discovery of another factor in the vitamin B complex. As early as 1912 Cooper [1912] showed that pigeons require to maintain their weight on a diet of polished rice a factor different in its distribution from that which protects against and cures polyneuritis, and it is possible that this factor was what is here designated vitamin B₂. But, since autoclaved yeast, one of the richest sources of vitamin B₂, has no effect in curing polyneuritis, it would follow that the presence or absence of vitamin B₂ in the food does not affect its strictly antineuritic potency. The distribution of the thermolabile antineuritic factor B₁, in so far as it has been investigated by experiments with birds fed on polished rice, has therefore not been upset by later discoveries.

Where the "water-soluble vitamin B" value of a substance has been investigated with the growth of rats as a criterion, it is not possible, since both factors are equally necessary for growth, to form any idea of the relative amounts of vitamins B₁ and B₂ present. Chick and Roscoe's [1928] work showed that it is difficult to clear caseinogen completely of vitamin B₂, and it may reasonably be assumed that most "vitamin B-free" diets have hitherto not been free of this factor. Moreover, it is not until recently that techniques have been evolved for supplying a rat with vitamin B₁ without vitamin B₂.

*Distribution of vitamin B₂ as indicated by previous observations
on the growth of rats.*

In the course of previous work demonstrating the dual nature of vitamin B, some indications of the distribution of vitamin B₂ have been given. This factor is abundant in yeast, fresh or autoclaved [Smith and Hendrick, 1926; Chick and Roscoe, 1927; Sherman and Axtmayer, 1927]; 0.2 g. daily of fresh yeast and 0.4 g. of autoclaved maintained growth in rats receiving daily an antineuritic yeast extract free from vitamin B₂ [Chick and Roscoe, 1927]. Sherman and Axtmayer [1927] found when observing growth in rats on a vitamin B-free diet that whole wheat supplemented autoclaved yeast, and dried skim milk supplemented whole wheat, and drew the conclusion that whole wheat is richer in vitamin B₁ (F) than in vitamin B₂ (G), while with milk the reverse is the case. Hunt and Kraus [1928] found that 5–10 cc. of fresh whole milk daily supplemented, as source of "vitamin B," a yeast-fraction which could also be supplemented by autoclaved yeast. A number of observations on pigeons have also indicated that milk is a poor source of the antineuritic vitamin [Cooper, 1914; Gibson and Conception, 1916; Johnson and Hooper, 1921].

Chick and Roscoe [1927] observed that it required a daily dose of 1 g. of wheat embryo to produce normal growth in conjunction with an antineuritic concentrate prepared according to Peters's method [Peters, 1924; Kinnersley and Peters, 1925], whereas 0.2 g. daily of the embryo could produce good growth when supplemented by autoclaved yeast.

McCollum, Simmonds and Pitz [1917] state, without giving experimental results, that the antineuritic value of the oat kernel is high; by analogy with other cereals one would expect this to be the case. Smith and Hendrick [1926], however, found that a diet containing 40 % of whole oats failed to produce normal growth in rats unless supplemented by 5 % fresh or autoclaved yeast, suggesting that oatmeal is richer in vitamin B₁ than in vitamin B₂.

Salmon [1927] obtained results suggesting that the leaves of the velvet bean were richer in vitamin B₂ than the beans themselves, while the distribution of vitamin B₁ was the opposite.

Goldberger *et al.* [1926] reported that rats fed on a diet containing 20 % of dried fresh beef as the sole source of vitamin B rapidly declined and died, with or without polyneuritis. 5 % of an alcoholic maize extract, which, supplemented by autoclaved yeast, could produce good growth, but of which alone as much as 40 % produced no growth, was added. With this addition the rats grew in a normal manner, suggesting that dried beef is a stronger source of vitamin B₂ than of vitamin B₁.

The results of these investigations can be roughly summarised as follows. Unheated yeast is rich in both vitamins B₁ and B₂; autoclaved yeast only in vitamin B₂. The cereals contain more vitamin B₁ than vitamin B₂, milk and meat the reverse. In the velvet bean the leaves are richer in vitamin B₂, the seeds in vitamin B₁. It is impossible to make any quantitative estimates of vitamin B₂ from these observations.

EXPERIMENTAL.

In the present research a thorough investigation of the wheat and maize kernels for their vitamin B₂ content was made; the point of interest being to determine, since human pellagra is a maize, rather than a wheat, eater's disease, any significant difference in vitamin B₂ content of the two cereals. We have also investigated certain other common foodstuffs in a less exhaustive fashion.

Method used for assaying vitamin B₂.

The technique elaborated by Chick and Roscoe [1928] was used. In this method B₁ is supplied to the rats as Peters's antineuritic concentrate, while special efforts are taken to free the caseinogen in the basal diet from vitamin B₂ by thorough washing with acidulated water and extraction with dilute acid alcohol. It is difficult to check all growth in rats and induce pellagra-like symptoms in them unless this method of purification is followed.

Our basal diet P₂L was as follows:

Specially purified caseinogen	20 parts
Rice starch	60 "
Cotton-seed oil	15 "
Salt mixture ¹	5 "
(Water)	100 ")

The diet was cooked in a steamer for 3 hours at 100° to prevent refection [Fridericia *et al.* 1927; Roscoe, 1927].

Diet P₂L, with the daily addition of 0.05–0.1 g. of cod-liver oil to provide vitamins A and D, and 0.1 cc. (equivalent to 0.6 g. yeast) daily of Peters's antineuritic concentrate [Peters, 1924; Kinnorsley and Peters, 1925], to provide vitamin B₁, was assumed to supply all the factors necessary for rat growth except vitamin B₂ (or the part of the vitamin B complex other than the antineuritic).

In the investigation of the cereals the materials under examination were mixed into the basal diet in such proportions that the standard ratio between protein, carbohydrate, and fat (20 : 60 : 15) was not altered, using for the calculation the commonly accepted analyses of the cereals. For example, white flour roughly contains: protein 10 %, carbohydrate 90 %; in constructing a diet containing 65 % of white flour, the following proportions were accordingly employed:

Purified caseinogen	13.5 parts
Rice starch	1.5 "
Cotton-seed oil	15.0 "
Salt mixture	5.0 "
White flour	65.0 " (6.5 parts protein; 58.5 parts carbohydrate)
(Water)	100.0 ")

making the percentages of protein, etc., similar to those in the basal diet P₂L. The composition of other cereal diets was similarly calculated. The diets were cooked for 3 hours at 100°. We did not in any case allow the cereal protein to replace more than one-half the caseinogen of the original basal diet; in most cases it replaced about one-third.

In the investigation of other foods, of which the necessary quantities were small, daily additions were made to the basal diet P₂L.

Young rats, just weaned, from 30 to 45 g. in weight, were fed for a week on diet P₂L, with the addition of three drops (0.05 g.) of cod-liver oil daily to provide vitamins A and D, Peters's antineuritic concentrate being omitted for economy's sake. At the end of the week, during which growth always ceased, the rats were put in separate cages and the diet containing the substance under examination was fed to them, 0.1 cc. (=0.6 g. dry yeast) daily of Peters's antineuritic concentrate (vitamin B₁) being now added. One rat from each litter, usually the best grown, was kept on diet P₂L with additions of cod-liver oil and antineuritic concentrate only, to act as a "negative control."

¹ Salt mixture No. 185 [McCollum, Simmonds and Pitz, 1917].

Young rats, when first caged singly and put on a strange diet, take a few days to get used to new conditions. Very little growth may take place in the first week, even on a good diet. We therefore disregarded the first week's growth, and took for our standard of comparison the average weekly growth during the succeeding 4 weeks, the vitamin B₂ value of a foodstuff being estimated by the minimum daily ration needed to maintain normal increase in body-weight (11-14 g. weekly) during those 4 weeks. In a few cases when, after a test period of 2 to 3 weeks, it became obvious that the diet contained insufficient vitamin B₂ for growth, we increased the amount of test substance in the ration and began again, using the succeeding 4 weeks for our standard of comparison.

We found sometimes a different response to the same diet by rats from different litters. This difference was not large enough to produce markedly incompatible results, but made it necessary to distribute each test material through several litters, so that in no case should conclusions be drawn from results obtained on rats of one litter only. Males and females were used in approximately equal numbers in testing each foodstuff.

Where it seemed of interest to compare as accurately as possible the vitamin B₂ content of certain foods, such as "household" and "top patent" flour and whole wheat and whole maize, rats from the same litter were used, as these give a more uniform response.

The power of certain foodstuffs to cure dermatitis which had developed in rats on the basal diet was also tested (Table V).

1. *The vitamin B₂ content of various samples of wheat, maize and peas, tested as different proportions of the diet.*

Two kinds of whole wheat were tested, (A) a mixture consisting mainly of Manitoba wheat, (B) a sample of English wheat. Of sample (A) five different parts of the berry were tested, viz. (1) the germ, which in this sample appeared to be quite free of bran, (2) the bran, (3) "top patent" flour, a sample specially collected and consisting of almost pure endosperm, (4) "household" flour, a coarser article than the "top patent" and one that is in commercial use, and (5) "tails" or fine pollard, a mixture of all parts of the berry carrying a high percentage of bran. The last was tested with the idea that it might represent a more or less pure specimen of the aleurone layer which lies between the bran and the endosperm, but on further investigation the sample was found to be too mixed for such conclusions to be drawn.

Two samples of whole maize were tested, (A) white African maize, (B) yellow South American maize. Maize germ meal and maize "grits" from sample (B) were also investigated. The maize germ meal contained a fair amount of endosperm, the maize "grits" was, as far as was ascertainable, fairly pure maize endosperm. "Polenta," another form of maize endosperm, was also tested.

In view of the extreme importance of the comparison of whole wheat and whole maize, an additional experiment was set up in which the two samples of whole wheat and the two samples of whole maize were tested on rats of similar weights from the same litter. The result of this experiment is shown in Fig. 1.

A sample of dried peas was tested.

The "polenta" and the peas were bought across the counter, the other samples being obtained direct from the mills.

The results of these experiments are shown in Table I, and in Table II are given the minimal amounts which must be present in the diet if normal growth is to be maintained.

Table I. *Average weekly growth increments of rats on: A, a mixed diet; B, purified diets, deficient in vitamin B₂, and with this vitamin supplied by autoclaved yeast; C, synthetic diets in which vitamin B₂ is supplied by various percentages of cereal products and peas.*

(Vitamin B₁ supplied in B and C by daily doses of Peters's antineuritic concentrate from yeast.)

Material tested		No. of rats observed	Average weekly increase in body-weight for chosen 4 weeks (g.)
A	Mixed diet. Dried or fresh winter milk, white or brown bread, cabbages or carrots. With Quaker oats, wheat bran, sheep's lungs and cods' heads once or twice a week	16	14 ♂ 16; ♀ 12.
		stock rats from 5th-9th week of life	
B	Negative controls. Diet P ₂ L	15	2
	Positive controls. Diet P ₂ L + autoclaved yeast (0.4 g. daily)	5	12.5
C	Wheat. Whole ground wheat. Sample A, Manitoba, 30 %	2	5.5
	50 %	10	16
	Sample B, English 50 %	4	12
	Wheat germ. Sample A, 7.5 %	4	2.5
	15 %	6	9
	30 %	6	16
	Wheat bran. Sample A, 15 %	4	8
	30 %	4	13.5
	Wheat "tails" (fine pollard). Sample A, 15 %	4	7.5
	30 %	4	15
	Flour. Top patent. Sample A, 65 %	7	5
	Household. Sample A, 65 %	4	5
	Maize. Whole ground maize. Sample A, white, African, 30 %	4	8.5
	50 %	6	11
	Sample B, yellow, S. American, 50 %	8	8
	Maize germ meal. Sample B, 15 %	4	2
	30 %	3	7
	60 %	6	13
	Endosperm. Maize "grits." Sample B, 65 %	6	5
	"Polenta," Italian, 65 %	4	4
	Peas. Dried ground "Clipper" peas, 30 %	2	9.5
	45 %	5	16

Whole wheat cannot be regarded as a rich source of vitamin B₂, since 30 % in the diet of sample A, a mixed sample consisting chiefly of Manitoba wheat, proved insufficient for growth; with 50 % of this wheat in the diet excellent growth was obtained. The sample of English wheat (B) was not as good a source of the vitamin; with 50 % in the diet growth was only just normal.

Wheat endosperm, either as "top patent" flour or as the less pure "household" flour, is a poor source of vitamin B₂, 65 % of either produced very little growth. Wheat embryo and bran are about equal to each other in their vitamin B₂ potency, and to wheat "tails"; in each case 15-30 % provides sufficient vitamin for normal growth. Tibbles [1912] gives the composition of the wheat berry as follows: bran 13.5 %, endosperm 85.0 %, germ 1.5 %, so that, in the wheat kernel as a whole the bran is the most important source of vitamin B₂.

Table II. *Minimum amounts of cereal products, given incorporated in the diet, required to provide enough vitamin B₂ for normal growth in young rats (11–14 g. weekly increase in body-weight).*

Material		Amount required % of diet
Wheat. B, English.	Whole grain	50
A, Manitoba.	Whole grain	30–50
	Embryo	15–30
	Bran	15–30
	Pollard	15–30
	Patent flour	> 65
	Household flour	> 65
Maize. A, white, African.	Whole grain	50
B, yellow, S. American.	Whole grain	> 50
	Germ meal	30–60
	Endosperm "grits"	> 65
C, endosperm Italian	"polenta"	> 65
Peas. Dried "Clippers"		30–45

N.B. Where two figures are given the lower was insufficient.

The mixed wheat sample, Manitoba predominating, was typical of the wheat mixtures used in London flour mills. It must be remembered, however, that since our investigation was made on particular samples, it is impossible to assume that these are representative.

Fig. 1. Comparison of growth-promoting value of two samples of whole maize and two of whole wheat, when given as the only source of vitamin B₂ in an otherwise complete diet. The curves show the average growth of two rats (1 ♂ and 1 ♀) on each diet. 50 % of the cereal product was included in the diet in each case.

Whole yellow maize is definitely inferior in its vitamin B₂ content to either of the samples of whole wheat tested. With 50 % in the diet the rats did not grow normally. The sample of white maize tested had a higher vitamin B₂ value than the yellow sample. The same amount, 50 %, in the diet was needed to produce normal growth as was needed of English wheat; it was, therefore, much inferior to the mixed Manitoba wheat (see Fig. 1).

Maize endosperm, as "grits" or as "polenta," is very low in the vitamin, as is wheat endosperm; with 65 % in the diet the rats scarcely grew at all. The maize germ meal used was a less cleanly milled product than the wheat germ. In the maize kernel the germ is attached to the endosperm by a large

scutellum and can only be prepared in the pure state by careful hand dissection. Maize germ meal, therefore, carries of necessity a large proportion of both endosperm and scutellum. Even taking this into account, the maize germ may be considered a poorer source of vitamin B₂ than wheat germ, as 60 % was needed to produce a normal rate of growth as against 30 % of wheat germ.

Vitamin B₂ and the antineuritic vitamin are differently distributed in the wheat and maize kernels. Vitamin B₂ is not entirely absent from the endosperm as is vitamin B₁. The rats on the 65 % white flour, and 65 % maize "grits," diets grew slightly better than those on the basal diet and did not develop pellagra-like symptoms except in one case, when a rat on 65 % "top patent" flour developed mild dermatitis. As regards the antineuritic vitamin B₁ "the germ, weight for weight, was found to be five times as potent as the bran in the cure of polyneuritis" [Medical Research Council, 1924]. In contrast with this, the vitamin B₂ content of the bran and the germ are about equal.

The vitamin B₂ value of dried peas cannot be considered high, 45 % in the diet being necessary before normal growth was obtained.

2. *The vitamin B₂ content of various foodstuffs, tested as additions to the basal diet.*

Yeast, dried and autoclaved for 5 hours at 120°, fresh whole winter milk from the ordinary London supply, cooked dried egg yolk, dried beef steak and dried ox liver were tested for their vitamin B₂ content by feeding small doses daily to rats which received the basal diet with the addition of Peters's yeast concentrate as source of vitamin B₁.

Table III. *Average weekly growth increments of rats on a purified caseinogen diet deficient in vitamin B₂ (P₂L), supplemented with daily doses of various foods.*

(Vitamin B₁ supplied by daily doses of Peters's antineuritic concentrate from yeast.)

Material tested	Amount given daily		No. of rats observed	Average weekly increase in body-weight for chosen 4 weeks (g.)
	Air dry g.	Natural state		
Liver (dried ox liver)	0.06	0.2 g.	2	4
	0.12	0.4	3	12
	0.25	0.8	1	18.5
Yeast (dry)	0.1	0.5 g.	2	8
	0.2	1.0	3	16
Yeast (autoclaved)	0.4	2.0 g.	5	12.5
Milk (fresh whole, London winter supply)	0.36)	3 cc.	3	9
	0.72) solids	6	3	12
	0.96)	8	2	14
Egg yolk (cooked, dried)	0.5	1 g.	2	7
	1.0	2	4	11
Meat (dried steak)	0.25	13	2	3.5
	0.5	2	3	9
	0.75	3	3	13
	1.0	4	2	15

The results of this experiment are given in Table III, and in Table IV are shown the minimum amounts which must be given if normal growth is to be maintained.

Table IV. *Minimum amounts of substances containing vitamin B₂ given as daily additions to a basal diet (P₂L), required for the normal growth of young rats (11–14 g. weekly increase in body-weight).*

Material	Daily dry weight g.	Daily amount as natural foodstuff
Liver (dried ox liver)	0.06–0.12	0.2–0.4 g.
Yeast (dried)	0.1–0.2	0.5–1.0 g.
Yeast (autoclaved)	0.4	2.0 g.
Milk (fresh whole, London winter supply)	0.36–0.72	3–6 cc.
Egg yolk (cooked, dried)	0.5–1.0	1–2 g.
Meat (dried steak)	0.5–0.75	2–3 g.

N.B. Where two figures are given the lower was insufficient.

All these natural foodstuffs enabled rats to increase normally in weight when given in doses of 1 g. and under.

Since we kept a daily record of the food consumption of each animal, it is possible to draw rough comparisons between the foods which were incorporated as different percentages of the diet and those fed as additions to it. Rats growing at a normal rate on diet P₂L (with additions) consumed from 6 to 7 g. (dry weight) daily. A dose of 1.0 g. dry weight would therefore represent 14–12.5 % of the dry weight of the total food consumed, one of 0.5 g. 7–6 %. Normal growth was obtained on the cereal diets only when 30 % or more of the cereal product was included in the total dry weight consumed, or when 2 g. and upwards of the cereal product was consumed daily.

The substances tested in Table III are therefore to be reckoned better sources of vitamin B₂ than the cereal products and peas, if the comparison is made on the dry weights of the substances concerned.

The following rough comparison of the foodstuffs tested may therefore be made, the substances being placed in descending order as regards their B₂ content, this being reckoned on the dry weight.

1. Dried ox liver	10. Dried peas
2. Dried yeast	11. Whole wheat (Manitoba)
3. Autoclaved yeast	12. „ „ (English)
4. Meat (dried steak)	13. Whole maize (white)
5. Milk solids	14. „ „ (yellow)
6. Dried egg yolk	15. Maize germ meal
7. Wheat germ	16. Wheat endosperm
8. „ bran	17. Maize endosperm
9. „ pollard	

3. *The curative effect of various foodstuffs on the dermatitis and other symptoms developing in rats on a vitamin B₂-deficient diet.*

Of 15 rats from 15 different litters kept as negative controls on diet P₂L with the addition only of cod-liver oil and antineuritic concentrate, none showed significant growth, and 9 developed symmetrical dermatitis. Of these 8 displayed lesions of the fore-paws, the hind feet also being affected in 6 cases and the groins and axillae in 3; one rat showed dermatitis of the groins and axillae alone. The axillary lesions tended to spread down the inner side of the forelimbs and across the chest and those in the groins down the inner side of the thighs. The dermatitis of the extremities was usually accompanied by oedema, which in 2 rats was so severe as to cause gangrene by constriction, with the loss of six toes in one case. The general condition of the rats showing skin symptoms was usually miserable.

The average time taken for symptoms to appear was 10 weeks, the shortest period being 5 weeks, and the longest 22.

The remaining 6 rats deprived of vitamin B₂, which showed no localised dermatitis, developed nevertheless a condition of general wretchedness after a period varying from 5 to 8 weeks. They showed a mixture of such symptoms as ophthalmia, inflamed nostrils, loss of hair on head and elsewhere, and haematuria. These symptoms were equally frequent in the rats showing definite dermatitis.

The symptomatology was as described in a previous paper from this laboratory [Chick and Roscoe, 1928], except that skin symptoms developed more constantly. A curious feature was the tendency of certain symptoms to predominate at certain times. At one period in the autumn the sick rats tended to develop severe lesions of the extremities and of the eyes, while later the groins and axillae were most often affected, the eyes being almost normal.

The power of certain foodstuffs to cure these symptoms of dermatitis was tested on eight of these negative control rats and on four other similar rats, negative controls from other experiments.

In addition to these young rats, two adult rats, aged about 1 year, were put on the vitamin B₂-free diet. One of these (801 ♀) dropped in weight from 350 to 170 g. in 11 weeks, and died without showing skin symptoms, though developing some ophthalmia. The other (802 ♂) dropped from 200 to 140 g. in 10 weeks, its weight then became stationary and at the end of 12 weeks it began to show dermatitis, which rapidly became very severe. This rat was used for a curative experiment.

The results of the curative experiments are given in Table V.

In these experiments it was found that the growth and healing properties of the substances tested ran parallel. The foodstuffs were mostly the same as those of which the vitamin B₂ content had been tested by growth experiments. The results confirm the idea that in the rat a growth-promoting and a dermatitis-preventing factor are identical. In addition to the substances used in the

Table V. *The curative effect of various substances on the dermatitis and other symptoms developing in rats on a vitamin B₂-deficient diet (P₂L).*

Rat	Condition before test	Substance tested	Length of time substance was fed	Result
771 ♀	Ophthalmia. Wretched condition. Weight stationary	Cheddar cheese 1 g. daily	3 weeks	Very slight improvement; 9 g. increase in wt.
714 ♂	Severe ophthalmia. Wretched condition. Dermatitis of extremities. Growth stationary	Cheddar cheese 2 g. daily	3 weeks	All lesions healed; 28 g. increase in wt.
711 ♀	Poor general condition. Dermatitis of axillae, groins and feet. Cessation of growth	Fresh meat 0.4 g. daily (0.1 g. dry wt.)	2 weeks	Improvement in dermatitis; 5 g. increase in wt.
734 ♀	Severe dermatitis of axillae and groins. Poor general condition. Cessation of growth, loss of weight	Cooked liver 0.4 g. daily (0.12 g. dry wt.)	3 weeks	Complete cure; 37 g. increase in wt.
3061 ♂	Severe dermatitis of fore-paws. Ophthalmia. Nose inflamed. Haematuria. Cessation of growth	Wheat germ 7½ %	1 week	No improvement or resumption of growth
		Wheat germ 15 %	1 week	Ditto
		Wheat bran 15 %	1 week	Ditto
		P ₂ L + 1.0 cc. yeast fraction ≡ 5 g. dry yeast	2 weeks	Complete cure; 28 g. increase in wt.
3067 ♂	Dermatitis of fore-paws. Inflamed nose. Haematuria. Cessation of growth	Patent flour 65 %	5 days	Died
378 ♂	Severe ophthalmia. Diarrhoea. Cessation of growth	Patent flour 65 %	3 weeks	Temporary improvement, followed by regression; no growth
		P ₂ L + 0.4 g. autoclaved yeast	3 weeks	Complete cure; 47 g. increase in wt.
728 ♂	Dermatitis of tail and extremities. Commencing gangrene of two toes. Cessation of growth	Maize germ meal 60 %	3 weeks	Toes sloughed off and all dermatitis healed; 30 g. increase in wt.
748 ♂	Severe dermatitis of fore-paws and feet. Commencing gangrene of toes. Dermatitis of tail. Weight stationary	Maize germ meal 60 %	3 weeks	Complete cure of dermatitis; toes sloughed off and stumps practically healed; 31 g. increase in wt.
729 ♂	Severe dermatitis of fore-paws. Weight stationary	Milk 6 cc. daily	3 weeks	All lesions healed; 38 g. increase in wt.
802 ♂	Great wasting. Dermatitis of axillae and groins, spreading down inside of thighs and forelimbs. Large raw area on abdomen	Egg-white 5 g.	3 weeks	Complete cure of all lesions; 31 g. increase in wt.
3075 ♀	Ophthalmia. Slight dermatitis of fore-paws. Cessation of growth	Egg-white 60 % of diet	3 weeks	Complete cure; 42 g. increase in wt.
757 ♀	Ophthalmia, very poor condition. Severe dermatitis of groins and axillae. Haematuria. Loss of weight	Egg-white extract (≡ 5 g. fresh egg-white)	4 weeks	Complete cure of all symptoms; 26 g. increase in wt.

growth experiments, Cheddar cheese and egg-white were tested. The former was not strongly curative as compared with milk; 2 g. daily of cheese (1.3 g. dry weight) was needed as compared with 6 cc. (0.7 g. dry weight) of milk. Egg-white was more potent; 5 g. (0.6 g. dry weight) produced a rapid amelioration of symptoms.

We have occasionally observed a very slow amelioration of symptoms taking place in rats kept throughout on the basal diet without any vitamin B₂ additions. In the above experiments, however, when improvement followed alterations in the diet it was striking and immediate and left little doubt of its cause.

Discussion of the relation of vitamin B₂ to the vitamin B complex, to the "P. P." (pellagra-preventive) factor, and to the factor preventing black-tongue in dogs.

It is by no means proved that vitamin B₂ is the only factor in the vitamin B complex other than that contained in Peters's antineuritic concentrate and several workers have brought forward evidence pointing to the existence of a third factor.

Hunt [1928] observed that an absorbable fraction (? vitamin B₁) and a filtrate (? vitamin B₂) from yeast, which could respectively supplement autoclaved yeast and whole wheat, could be further supplemented by whole fresh yeast or yeast residue minus the above fractions. We do not think, however, that in these experiments the possibility of a quantitative deficiency of either fraction was sufficiently excluded. The same criticism applies to Reader's observations [1928] that growth failed in rats whose vitamin B was supplied by Peters's antineuritic concentrate and large amounts of autoclaved marmite. Chick and Roscoe [1927] find that Peters's yeast concentrate in an amount equivalent to 0.6 g. yeast daily if supplemented by 0.4 to 0.6 g. autoclaved yeast supports normal growth.

We ourselves are more concerned with the possibility that in our experiments lack of growth and skin symptoms may not be due to the absence of the same factor. We can only say that those foods which promoted fair or good growth in young rats also protected from skin symptoms, and that in the curative experiments growth and healing ran parallel. The fact, however, that vitamin B₂ may be capable of further subdivision would not preclude its identity with Goldberger's pellagra-preventive "P. P." factor, itself possibly dual. In man two distinct conditions are described: pellagra, and *pellagra sine pellagra*, in which no skin eruption occurs. The "P. P." factor is effective against both.

In our experience the distribution of vitamin B₂ as determined by the method given above corresponds closely with that of the "P. P." factor and of the factor which prevents black-tongue in dogs, investigated by Goldberger, Voegtlin and others. The estimations made by these workers of the "P. P." factor contained in various foods are approximate, since in few cases was any attempt made to estimate the minimum necessary for protection, and the

basal diet was necessarily varied. Their results are collected in Table VI, the amounts given being expressed as the percentage of the dry weight of the diet, in order to obtain a rough comparison with our own results.

Table VI. *Comparison of the effects of various foodstuffs on vitamin B₂ deficiency in rats, on pellagra in man, and on black-tongue in dogs.*

Material	Rat Vitamin B ₂	Man P.P. factor	Dog Black-tongue preventive factor
Maize endosperm	Very poor	Associated with pellagra in U.S.A. and Italy	—
Wheat flour	Very poor	Associated with pellagra in U.S.A.	Used in basal diet to produce the disease
Whole maize	Poor; 50 % less than normal growth	Traditionally associated with pellagra	No preventive action, used in basal diet to produce the disease
Whole wheat	Poor, but better than maize	—	66 % in diet delayed onset of the disease; no protection
Peas	30–40 % needed for good growth ("Clipper" peas)	30–35 % (168 g. daily); incomplete protection (Virginian cowpea)	58 %; slight protection, 73 %; almost complete protection (Virginian cowpea)
Wheat germ	15–30 % good growth	30 %; protection almost complete	31 %; protection almost complete
Fresh milk	5–10 % milk solids, good growth	20 % solids (1200 g. buttermilk daily) protected	250–300 cc. skim milk daily protected
Lean meat	7–10 % (dry wt.) good growth	10 % (dry wt., 50 g. daily) protected	11 % (dry wt.) protected
Dried yeast	1–3 % good growth	7 % (30 g. daily) protected	5 % protected
Liver	1–2 % (dry wt.) ox liver, good growth	Alcoholic extract of ox liver effective in treatment	11 % dried pork liver protected
Dried egg yolk	7–15 % good growth	—	17 % protected

Dried yeast is strongly preventive of pellagra; 30 g. daily averted the disease in asylum patients, otherwise liable [Goldberger and Tanner, 1925]. Fresh or autoclaved, it is also effective against black-tongue, if present in the proportion of 2–5 % of the basal diet [Goldberger *et al.*, 1928, 1].

Goldberger's original field studies in pellagra [Goldberger, Wheeler and Stucker, 1920] showed that "increasing supplies of milk or fresh meat were associated, one independently of the other, with a decreasing pellagra incidence," and later work has demonstrated the protective value of both foods against pellagra and against black-tongue [Goldberger *et al.*, 1924, 1926, 1928, 2]. Egg yolk, not yet tested in human pellagra, averts the canine disease [Goldberger *et al.*, 1928, 2]. Cowpeas gave some protection against both pellagra and black-tongue [Goldberger *et al.*, 1927, 1928, 2], but were not reckoned a good source of the protective factors.

An 85 % alcoholic extract of pork liver was used with good effect in the treatment of pellagra by Voegtlin [1920, Voegtlin *et al.*, 1920]. It was claimed

that a daily therapeutic dose equal to 1 kg. of fresh liver produced as good an effect as "a diet containing a considerable quantity of milk, eggs and meat." Dried pork liver is also preventive of black-tongue [Goldberger *et al.*, 1928, 2]. We found dried ox liver to be the most potent source of vitamin B₂ among the substances tested.

We found a mixed whole wheat, in which Manitoba wheat predominated, to have more vitamin B₂ potency than white or yellow maize. If vitamin B₂ and the "P. P." factor are identical, this result might explain the observed liability to pellagra of the maize eater rather than of the wheat eater, in localities where the whole grain and not highly milled products are consumed. Similarly, studies on black-tongue [Goldberger *et al.*, 1928, 1, 2] showed that the appearance of symptoms was earlier when the basal diet contained 78 % whole maize than when it contained 50 % whole wheat.

The endosperm of these two cereals, white flour and maize "grits," we found, on the other hand, to be about equally low in vitamin B₂ content. Therefore, if this vitamin be held to be a preventive of pellagra, white flour and maize endosperm should be equally conducive to the disease. This is possibly the case. Goldberger, Wheeler and Stucker [1920], in their field studies, found, when considering the poorest households with low supplies of fresh meat and milk, that no regular inverse ratio could be made out between the incidence of pellagra and the amount of white flour consumed. A rise in the household supply from 3 to 16 lbs. per adult male over a 15-day period did not cause any regular fall in the percentage of households affected by the disease. In the first report of the Thompson, McFadden Pellagra Commission [Siler, Garrison and MacNeal, 1913] it was stated that in the pellagra-affected districts studied, "wheat flour, in the form of bread or biscuit, is the principal bread-stuff, and corn-meal, while extensively used, is not nearly so staple an element of the dietary as wheat flour." It is possible that, where it is the custom to eat highly milled cereals, as in the United States, the more frequent association of pellagra with a maize than with a wheat diet is accidental, depending on other deficiencies in the diet of the maize eater. A maize diet is usually associated with poverty and marked by a shortage of such expensive foodstuffs as milk, meat and eggs.

Pellagra seems to be a disease of recent origin in the United States. According to Wood [1909], only sporadic cases were reported before 1909, and modern methods of milling are often held to be responsible for its recent increased prevalence. Our results support this to some extent, at any rate as far as wheat is concerned, since of all the cereal products examined we found wheat bran and wheat embryo richest in vitamin B₂.

Here again our results conform with those of Goldberger and his colleagues [1927, 1928, 2] on pellagra and black-tongue. A diet containing 60 % of maize germ meal cured two rats suffering from severe dermatitis, but since only poor growth occurred with a diet containing 30 %, maize germ meal must be considered a low source of the vitamin. Wood [1920] claimed to have fed

pellagrins on maize germ with prompt improvement. In our own experiments whole yellow maize as source of vitamin B₂ gave better growth than yellow maize "grits" (endosperm), showing that vitamin B₂ is lost in milling. This is more definitely the case with wheat, and it may be that the onset of pellagra in the United States coincided with the introduction of standard highly milled wheat and maize flour.

Evidence of the identity of vitamin B₂ deficiency in rats, of pellagra in man, and of black-tongue in dogs, is given in Table VI. The substances tested for the pellagra-preventive factor by Goldberger and his colleagues were given as daily additions to a daily diet of 400–500 g. (dry weight), equal to 2000–2500 calories. In the black-tongue experiments the test substances were incorporated in dietary mixtures of 400–500 g. (dry weight). From the tables given by these workers the percentages of the test substances in the diet have been roughly calculated.

There is very fair agreement in the effects of the various foods on the three conditions, and there seems little doubt that the condition produced in rats by a diet deficient in vitamin B₂ is the analogue of human pellagra. We suggest that our method might be useful in tackling any problems in the human disease that remain to be solved.

It is worth noticing that the rat, as compared with man and the dog, is relatively insusceptible to pellagra. Diets that produce florid pellagra in man and black-tongue in dogs do not cause dermatitis in rats, and only a rigorous purification of the basal diet can ensure the appearance of symptoms.

SUMMARY.

(1) The method previously described by Chick and Roscoe [1928] has been used for estimating the vitamin B₂ value of foodstuffs. The special features of this method are (a) elaborate purification of the caseinogen in the basal diet, and (b) the use of Peters's antineuritic concentrate as source of vitamin B₁.

(2) The vitamin B₂ value of a substance was estimated by the minimum amount required to support normal increase in body-weight (11–14 g. per week) in young newly weaned rats receiving, apart from the test substance, a diet complete in all respects except vitamin B₂. For one week previous to the test the rats were maintained on a diet entirely free of both the B vitamins.

(3) Control rats from each litter used were fed on the basal diet, complete except for vitamin B₂. All remained stationary in weight and the majority developed skin symptoms.

(4) Some curative experiments on such rats suffering from dermatitis were carried out with materials containing vitamin B₂. In these amelioration of symptoms and resumption of growth were parallel.

(5) The vitamin B₂ value of wheat and maize is poor, that of maize being on the whole the lower in the samples examined. In wheat the germ and bran are better sources than the endosperm, and about equal to each other. In maize the germ is not so rich a source, but whole maize is better than maize endosperm.

- (6) Dried peas have a low vitamin B₂ content.
- (7) Dried ox liver, yeast and fresh whole milk are excellent sources of vitamin B₂. Dried meat and egg yolk are less good but richer than cereals.
- (8) The relation of vitamin B₂ to Goldberger's "P. P." factor and to the factor preventing black-tongue in dogs is discussed. It is noted that so far the distribution of all three has been found to be identical.
- (9) Some problems in the epidemiology of pellagra are discussed on the assumption that vitamin B₂ is identical with Goldberger's pellagra-preventive factor.

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