CXXXIV. THE DISTRIBUTION OF THE VITAMIN B COMPLEX.

II. ROOT VEGETABLES.

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(Received July 1st, 1931.)

THE following work is a continuation of that already carried out in this laboratory on the estimation of the amount of the antineuritic vitamin B₁ and the antidermatitis vitamin B₂ in naturally-occurring foodstuffs [Aykroyd and Roscoe, 1929; Aykroyd, 1930; Roscoe, 1930].

Since this work was started several constituents of the vitamin B complex other than vitamins B₁ and B₂ have been differentiated by various workers. Vitamin B₃ [Williams and Waterman, 1928] has been shown to be necessary for the growth of pigeons but not for that of rats, on which these experiments have been carried out. Vitamin B₄ has been shown by Reader [1928, 1929, 1930] to be necessary for the continued growth and well-being of rats, and the possibility of its presence or absence having affected the assays of vitamins B₁ and B₂ described in this and former papers is discussed later.

HISTORICAL.

The presence of the antineuritic vitamin (B₁) in the potato was established by the early workers with pigeons. Vedder and Clark [1912] obtained partial prevention of polyneuritis with 10 g. of the fresh tuber. McCollum and Kennedy [1916] obtained a slow cure with potato juice and Chick and Hume [1917] with an alcoholic extract of the peelings. An extract of the peeled tuber improved the condition of the birds but did not cure the nervous symptoms.

A number of American workers tested various root vegetables by feeding them to rats as the sole source of the vitamin B complex [McCollum, Simmonds and Parsons, 1918; Denton and Kohman, 1918; Steenbock and Gross, 1919]. None of these tests, however, can be interpreted to show minimal amounts of the roots providing adequate supplies of either vitamin B₁ or B₂, since it must remain uncertain which of the two was acting as limiting factor. Moreover the vitamin-containing substances were fed as percentages of the diet and as will be shown later this method is not capable of quantitative interpretation.

Osborne and Mendel [1919, 1920] investigated the daily dose of roots

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necessary for the normal growth of rats when supplementing a basal diet, the protein of which was an extracted meat residue. It seems probable from control experiments [Osborne and Mendel, 1917] that this residue contained vitamin B₂ but no vitamin B₁, and that their experiments may, therefore, be interpreted as vitamin B₁ estimations. They found that 1.0 g. daily dry weight of either carrot or turnip was needed for good growth, 1.0 g. dry weight of either old or new potato only provided enough vitamin for subnormal growth, 2.0 g. was enough for approximately normal growth. The peel was not found to be a better source of this vitamin than the inside. 2.0 g. of beetroot supported fairly good growth.

Dunham [1921] used the weight maintenance of 4-5 weeks old rats as criterion of "vitamin B" content. Since vitamin B₂ is not necessary for weight maintenance his results may also be interpreted as vitamin B₁ estimations. He found that 0.065 g. of dry potato, 0.07 g. of dry carrot, or 0.105 g. of turnip was needed. The lowness of his figures, as compared with those of Sherman given below, is probably due to the fact that in his experiments coprophagy was not prevented.

Sherman [1926] also quotes figures for the weight maintenance of young rats, but no experimental details are given. He reports that 3.2-3.8 g. fresh (0.32-0.38 g. dry) of carrot and 3.5 g. fresh (0.38 g. dry) of turnip and 3.5 g. fresh (0.7 g. dry) of potato were needed to maintain weight.

Morgan [1924] obtained cures of paralysis due to vitamin B₁ deficiency in rats by daily doses of 1.0 g. of dry radish. The cures and resultant growth were not quite so good as those with rats receiving 0.3 g. of dry brewer's yeast.

In the following paper the vitamin B₁ and vitamin B₂ values of potato, carrot and turnip are reported and compared with those of yeast and the other natural foodstuffs previously investigated. These vegetables were chosen as representative species of the so-called root vegetables, the potato being really an underground stem or tuber. Carrots and turnips are both roots but they differ in that the carrot is highly pigmented and the turnip is colourless. All three are among the most commonly used in ordinary diets.

METHODS.

The methods employed were those described in the previous paper [Roscoe, 1930], the growth of young, 40 g., rats being used as criterion. These methods are slight modifications of those of Chick and Roscoe [1928, 1929].

The vitamin B₁ estimations were made using the unpurified caseinogen diet (FL), vitamin B₂ being supplied as autoclaved yeast extract (F5A). The vitamin B₂ estimations were made with the purified caseinogen diet (P2L) with vitamin B₁ as Peters's antineuritic concentrate from yeast (YE) [Peters, 1924; Kinnersley and Peters, 1925].

The relatively large amounts of the roots that it was necessary to administer made it impracticable to give them as daily doses. They were therefore minced and incorporated in the diet, the caseinogen and rice starch of which were varied so as to keep the percentage of protein at 20, the rest of the material being substituted for rice starch. The rats were not, however, allowed to eat more than a given amount of these diets, the remainder of their requirements being made up with FL or P2L.

Diets with the following varying percentages of incorporated vegetables were employed:

10 %	daily ration	2.5 g.	equivalent to a daily dose of	0.25 g.
10 %	"	5.0 g.	"	0.5 g.
20 %	"	5.0 g.	"	1.0 g.
40 %	"	5.0 g.	"	2.0 g.
65 %	"	6.0 g.	"	4.0 g.

These figures are for dry weights. All the diets were, however, mixed with 50 % of water. When the roots contained more water than would make up this amount they were reduced before incorporation in the diet by partial drying at 37°. All diets were cooked for 2-3 hours.

THE VITAMIN B₁ CONTENT OF ROOT VEGETABLES.

The results of these experiments are given in Table I. The amounts consumed daily, given in the second column, are expressed as dry weight.

Table I. *Vitamin B₁ content of root vegetables as demonstrated by weight increase of rats during five weeks on a diet, complete, except for vitamin B₁, and containing various amounts of the root vegetables to be tested.*

Material tested	Amount eaten daily, g. dry weight	No. of rats	Increase in weight in 5 weeks (averages, g.)
Carrot	0.25	6	27
	0.5	4	65
	1.0	4	88
Turnip	0.5	4	49
	1.0	5	79
Potato	1.0	4	45
	2.0	4	95

Carrot. This proved a good source of the antineuritic vitamin; rats receiving 0.5 g. gained 65 g. in weight in five weeks, those receiving 1.0 g., 88 g.

Turnip. This was a slightly less good source; rats receiving 0.5 g. gained 49 g. in five weeks, those receiving 1.0 g., 79 g.

Potato. This was a poor source of the vitamin; rats receiving 1.0 g. only increased in weight 45 g. in 5 weeks, those receiving 2.0 g. increased 95 g.

In Table III the amounts of these three root vegetables and of other food-stuffs previously tested needed to give 50-60 g. increase in weight in rats in 5 weeks when given as source of vitamin B₁ are compared and their relative potency is given, yeast being taken as 100.

It will be seen that carrot and turnip are much less potent than yeast in vitamin B₁, approximately equal to the green leafy vegetables and better than egg-yolk or meat. Potato is poorer than any of these.

Potato starch was also tested for its vitamin B₁ content, but the animals would not eat enough to provide a dose of more than 2-3 g. daily and these collapsed with paralysis at the same time as the controls. When receiving a small dose of vitamin B₁ extract, however, the animals on a diet containing potato starch grew better than those on a diet containing rice starch. There would therefore appear to be a small amount of the antineuritic vitamin in potato starch.

THE VITAMIN B₂ CONTENT OF ROOT VEGETABLES.

The results of these experiments are given in Table II. The amounts consumed daily are expressed as the dry weight.

Table II. *Vitamin B₂ content of root vegetables as demonstrated by weight increase of rats during five weeks on a diet, complete, except for vitamin B₂, and containing various percentages of the root vegetables to be tested.*

Material tested	Amount eaten daily, g. dry weight	No. of rats	Increase in weight in 5 weeks (averages, g.)
Carrot	0.5	6	48
	1.0	4	67
	2.0	4	81
Turnip	1.0	4	52
	2.0	4	62
Potato	2.0	4	38
	4.0	4	71

Carrot. This proved the richest of the root vegetables tested in vitamin B₂ as in vitamin B₁; rats receiving 0.5 g. gained 48 g. in 5 weeks, those receiving 1.0 g., 67 g., and those with 2.0 g., 81 g.

Turnip. This was less rich; rats receiving 1.0 g. gained 52 g. and those receiving 2.0 g., 62 g.

Potato. This was a poor source of the vitamin; rats receiving as much as 2.0 g. gained only 38 g. but those with 4.0 g. gained 71 g. during the five weeks of the experiment.

In Table III these vegetables and other foodstuffs are compared with yeast

Table III. *The vitamin B₁ and B₂ content of various foodstuffs compared with that of yeast.*

In columns 1, 2, 4 and 5 are given the minimal amounts needed to promote an increase in weight of 50–60 g. in 5 weeks in young rats of 40 g. weight when given as source of vitamins B₁ and B₂ respectively.

In columns 3 and 6 are shown the relative potencies of the dried foodstuffs when yeast is taken as 100.

Material	Water content (%)	As source of vitamin B ₁			As source of vitamin B ₂		
		Weight increase		Potency Dry wt.	Weight increase		Potency Dry wt.
		Wet wt.	Dry wt.		Wet wt.	Dry wt.	
		1	2	3	4	5	6
Yeast	80	0.25	0.05	100	0.5	0.1	100
Green vegetables	90	3.0–5.0	0.3–0.5	10–17	4.0–6.0	0.4–0.6	17–25
Carrot	90	2.5–5.0	0.25–0.5	10–20	>5.0	>0.5	<20
Turnip	93	>7.0	>0.5	<10	14.3	1.0	10
Egg-yolk	50	<2.0	<1.0	>5	2.0	1.0	10
Meat, steak	75	4.0	1.0	5	2.8	0.7	14
Milk	88	—	—	—	6.0	0.7	14
Potato	78	5.0–10.0	1.0–2.0	<5	10.0–20.0	2.0–4.0	<5

as regards their vitamin B₂ potency, on the basis of the amounts needed of each to promote 50–60 g. increase in weight in 5 weeks.

All are of much lower potency than yeast; carrot has a potency equal to

that of milk, meat and the less good green vegetables, but better than egg-yolk or wheat germ. Turnip is equal to the latter group. Potato is of the same order of strength as the cereals and pulses.

Potato starch was also tested for its vitamin B₂ content. Two rats receiving 60 % potato starch diet and eating such an amount that they received an average of 2.2 and 2.6 g. of starch daily grew better than the controls in the same litter though not better than controls sometimes grow. A very low content of this vitamin is indicated.

Experiments were carried out in which these vegetables were used in the cure of dermatitis arising from a deficiency of vitamin B₂. 0.5 g. of dry carrot, turnip or potato was found to cure symptoms. The minimal amounts needed were not discovered.

The above experiments were carried on for 5 weeks only, but additional experiments were carried out in which the growth to normal adult weight, when the vegetable was the sole source of the vitamin B complex, was investigated.

Three rats, a male and two females, when receiving 2.0–3.0 g. daily of dry carrot followed the normal growth curve. A litter was born to each of the females but only one of the young survived: this one was reared to weaning.

Similarly with 4.0–8.0 g. of potato normal growth curves were obtained and each rat produced a litter. One litter of six was reared to weaning, the other, however, was not fed.

Continued breeding was not investigated.

THE EFFECT OF THE AGE OF THE ROOT ON THE VITAMIN B CONTENT.

A comparison of the vitamin B₁ and vitamin B₂ contents of old carrots which had been stored all winter with those of fresh spring carrots was made. No significant difference was observed. In the vitamin B₁ tests, rats which received 0.25 g. of old carrot increased in weight an average of 29 g. in the 5 weeks of the experiment, those receiving the new carrot 20 g. In the vitamin B₂ tests those receiving 0.5 g. of old carrot increased 42 g. in weight, those with the same addition of new carrot 44 g.

This result recalls that of Osborne and Mendel [1920] who found new potatoes not to be superior in "water-soluble B" to old ones.

THE EFFECT OF COOKING ON THE AVAILABILITY OF THE B-VITAMINS.

In the previous experiments the cooking of the diets in which the vitamin-containing substances were included involved the steaming of the vegetables for 2–3 hours. It was thought possible that this cooking might render them more digestible and so increase the degree of absorption of the B-vitamins from the alimentary canal of the rat. An experiment was therefore performed in which the same quantities of carrot were fed (i) as a percentage of the diet which was cooked for 2–3 hours and (ii) as daily doses of the uncooked vegetable. Vitamin B₁ and vitamin B₂ tests were carried out in this way.

No difference was observable between the two groups. In the vitamin B₁ tests the average increase in weight in 5 weeks with 0.25 g. of cooked carrot was 31 g.; with the same amount of uncooked carrot it was 29 g. In the vitamin B₂ tests with 0.5 g. of cooked carrot there was an increase in weight of 47 g., with the uncooked of 42 g.

THE VITAMIN B₄ CONTENT OF THE VEGETABLES.

Vitamin B₄ deficiency in rats is characterised by failure to grow after about 4 weeks of deprivation and in acute stages by listlessness, a hunched attitude, and inflamed and swollen paws [Reader, 1928, 1929, 1930]. These symptoms have been observed in these experiments among the negative control rats which were receiving vitamin B₂ as autoclaved yeast extract together with a diet containing unpurified caseinogen. It is therefore clear that vitamin B₄ is not present in the basal diet or in the source of vitamin B₂ given in these vitamin B₁ tests. Thus it is possible that these and previous vitamin B₁ estimations may have been affected by a deficiency of vitamin B₄.

Vitamins B₁ and B₄ are, however, very closely related and there is as yet no evidence that they have a different distribution in nature. Moreover in the experiments here reported and in the majority of the previous experiments, normal growth (14–18 g. per week) was obtained with the higher doses at a time when the controls were showing symptoms of vitamin B₄ deficiency; in these experiments therefore vitamin B₄ must have been present in adequate amounts.

A further test was made in which rats on small doses of carrot, after several weeks of subnormal growth, received in addition the preparation of vitamin B₄ described by Reader [1930]. No improvement in growth was observed, showing that the limiting factor was not a deficiency of vitamin B₄.

It may therefore be taken as proved that vitamin B₄ was present in amounts sufficient for normal growth in the vegetables here tested.

In the vitamin B₂ tests vitamin B₄ is present in adequate amounts in the antineuritic yeast concentrate made according to the method of Peters, the charcoal being extracted with acid alcohol.

DISCUSSION.

The vitamin B₁ and vitamin B₂ contents of the root vegetables appear from these results to vary considerably, carrot being a good source of both the vitamins, potato a poor one and turnip slightly poorer than carrot.

The proportion of vitamin B₁ to vitamin B₂ is similar to that found in the majority of the leafy vegetables, twice as much of the material being needed for good growth when given as source of vitamin B₂ as when given as source of vitamin B₁. This is the same proportion as is also found in brewer's yeast.

These results confirm the conclusions of all investigators in showing that carrot is a fairly good source of the B-vitamins and potato a poor one, and more

particularly they agree with those of Osborne and Mendel [1919, 1920] in showing that 1.0 g. of dry carrot and 2.0 g. of potato contain sufficient vitamin B₁ for normal growth of rats. In disagreement with the present work these authors claimed that turnip was as good a source of the antineuritic vitamin as carrot. Beyond this it is difficult to compare the results here obtained with those of previous workers.

The practice of administering the vitamin-containing substance as a percentage of the diet which is freely consumed by the experimental animal, makes the interpretation of many early results very difficult. In vitamin B₁ tests particularly this method is very uncertain in its results.

In such experiments it is possible by careful measurement of the food consumed to assess the daily intake of the vitamin-containing substance, but it is not possible, except by limiting the total intake, to insure that each animal on a given diet shall eat the same amount. Aykroyd has shown (unpublished results) that on a test diet containing a percentage of a substance low in vitamin B₁ some rats may consume only a small amount of food, and as a result do not obtain enough vitamin B₁ to protect them against paralysis, while others eat about twice as much of the diet and thereby acquire enough of the vitamin to increase their weight by 10–13 g. a week. In fact in one case a rat at first grew 10 g. a week and then ceasing to eat well lost weight and developed paralysis.

This fact would seem to disprove, as far as vitamin B₁ is concerned, the theory put forward by Plimmer, Rosedale and Raymond [1927] that the amount of vitamins B₁ and B₂ (no distinction between the two vitamins is made by these authors) needed is directly proportional to the calories consumed. For in experiments such as the above where the vitamin-containing substance is given as a percentage of the diet the proportion of vitamin to calories ingested is constant whatever the amount eaten, yet the deficiency of a rat which develops paralysis must be considered greater than that of one which shows an increase in weight of 10–13 g. a week.

A further fact indicating that the need for either vitamin B₁ or vitamin B₂ is not dependent on the calorie intake is that at the end of the five weeks tests for either of these vitamins the rats were in some cases eating twice as much food as at the beginning, yet although their vitamin supply had not been increased their growth was just as good.

SUMMARY.

1. The vitamin B₁ content of carrot is about one-fifth that of yeast (dry weights), equal to that of the less good leafy vegetables and better than that of egg-yolk or milk. Turnip is not quite so rich in the vitamin, and potato is poor, being only one-fifteenth as rich as yeast.

2. Carrot is one-fifth as good a source of vitamin B₂ as yeast and about equal to milk, meat and the less good leafy vegetables. Turnip is less potent,

having the same potency as egg-yolk and wheat germ. Potato has a low content of this vitamin, comparable with that of the cereals and pulses, but the relatively large amounts of this vegetable eaten may render it an important source of both the B-vitamins in an ordinary diet.

3. Potato starch contains small amounts of both vitamins, B₁ and B₂.
4. New spring carrots contain no more vitamin B₁ or B₂ than do old ones which have been stored all winter.
5. The vitamins B₁ and B₂ in carrots are not rendered more available to the animal consuming them by steaming for two to three hours.
6. Reasons are given indicating the presence of vitamin B₄ in the vegetables tested.
7. The method of testing for the B-vitamins by incorporation of the test material in the basal diet is criticised and it is pointed out that this method is not admissible unless the consumption of the diet is limited.
8. The vitamin B₁ requirements of the rat do not seem to be related to the total amount of food consumed.

My thanks are due to Dr H. Chick for advice and criticism throughout the course of this work, and to Miss D. Graetz for help with the experimental animals.

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