CXCVII. THE DISTRIBUTION OF THE VITAMIN B COMPLEX.

I. LEAFY VEGETABLES.

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THE work described in this paper is a continuation of that already carried out in this laboratory [Aykroyd and Roscoe, 1929] on the distribution among foodstuffs of the two constituents of the vitamin B complex, vitamins B₁ and B₂¹.

The greater part of the early work on the distribution of vitamin B needs reconsideration since the differentiation of the complex into the two constituents, vitamins B₁ and B₂. Some deductions can however be made from this work.

The classic work on the antineuritic vitamin, using pigeons and fowls, remains uncomplicated by any considerations of vitamin B₂ deficiency, since it is concerned purely with the cure and prevention of polyneuritis. Thus both McCollum and Kennedy [1916] and Chick and Hume [1917] showed that preparations from cabbage will cure polyneuritis in pigeons. But no quantitative results can be drawn from their figures.

Osborne and Mendel [1919, 1, 2; 1920] in their investigation of the vitamin B content of green vegetables used as protein an extracted meat residue, which from their control experiments [Osborne and Mendel, 1917] appears to have contained vitamin B₂, though this was not then recognised, but to have been devoid of vitamin B₁. Their results can therefore be interpreted as an estimation of vitamin B₁. They found that 0.5–1 g. of alfalfa, clover or spinach, 1 g. of cabbage or more than 1 g. of timothy grass, all reckoned on the dry weight, was necessary daily to promote the growth of young rats, 0.2 g. being the amount of dry yeast needed.

Dunham [1921] and Sherman [1926] used maintenance, not gain in weight, as their criterion. Since vitamin B₂ is not necessary for weight maintenance, their results may also be interpreted as vitamin B₁ estimations. Sherman found that 0.1–0.15 g. dry weight of spinach, 0.15–0.3 g. of cabbage, 0.2–0.3 g. of lettuce or 0.3 g. of onion was needed daily for the weight maintenance of young rats. The figures given by Dunham were lower, viz. 0.07 g. for cabbage and 0.085 g. for spinach, but, as coprophagy was not prevented, the rats probably acquired some vitamin B from their faeces.

¹ For the nomenclature used in this paper see footnote, p. 1764.

Other workers give estimates of vitamin B which can only be interpreted as showing amounts, not necessarily minimal, which provided adequate quantities of both vitamins B₁ and B₂ for the normal growth of the young rat. Thus rats grew normally when the following amounts (dry weight) were fed: 20 % of cabbage in the diet [Daniels and McClurg, 1919], daily doses of 0.4 g. of lettuce and parsley, more than 0.6 g. of dandelion leaves, 1.0 g. of onion and 1.0–2.0 g. of beet leaves [Osborne and Mendel, 1919, 2; 1922], 0.5–2.0 g. of spinach [Eddy, Kohman and Carlsson, 1925], and 0.4 g. of watercress [Mendel and Vickery, 1929].

Salmon [1927] distinguished between vitamin B₁ and vitamin B₂, using pigeon maintenance and rat maintenance in his vitamin B₁ estimations, and rat growth in his vitamin B₂ estimations. The latter observations may therefore be influenced by the vitamin B₁ content, but with the green leaves he tested it is doubtful if this was the case. He found that a daily dose of the equivalent of 0.35–0.4 g. dry weight of rape or velvet bean leaves was sufficient for maintenance of the pigeon, 0.1–0.15 g. dry weight of rape leaves lengthened the life of the rat and 0.25 g. dry weight of velvet bean leaves doubled it. 1–2 g. dry weight of rape leaves or velvet bean leaves was sufficient for good rat growth as against 0.4–0.5 g. of dry yeast.

These results indicate that the vitamin B₁ and vitamin B₂ values of the leafy vegetables are both high, being from one-half to one-fifth that of yeast, when dry weights are considered.

In the present paper vitamin B₁ and vitamin B₂ estimations of watercress, lettuce, cabbage, spinach and onion are reported.

METHODS.

The methods employed were those described by Chick and Roscoe for vitamin B₁ [1929] and vitamin B₂ [1928] in which the criterion was the growth of the young rat. The rats received the experimental diet when just weaned and weighing about 40 g. They were fed for a preliminary week on a basal diet (P2L, Table I) deprived of both B vitamins.

Vitamin B₁. The estimations of vitamin B₁ were made in duplicate, using the two vitamin B₂-containing diets previously described by Chick and Roscoe [1929].

In one (EL, Table I) the protein and vitamin B₂ were provided by egg-white; in the other (FL) the protein was supplied as caseinogen and the vitamin B₂ as an autoclaved yeast extract (F5A, Table I). The diet FL differed from that used by Chick and Roscoe (P2L, 1929) in the substitution of "light white casein" (B.D.H.) for the highly purified caseinogen. It was found, following the work of Coward *et al.* [1929], that the former product contained no appreciable amounts of vitamin B₁.

The autoclaved brewer's yeast used previously as source of vitamin B₂ was found to have two disadvantages. It was apt to cause diarrhoea, and some batches, although autoclaved under apparently similar circumstances, appeared to contain small quantities of vitamin B₁, with the result that the time taken by the rats to become depleted of their stores of this vitamin was uncertain and sometimes long. In the present work vitamin B₂ was therefore given as daily doses, equivalent to 0.5 g. of dry yeast, of an extract made from washed brewer's yeast by boiling with 0.01 % acetic acid and autoclaving for 5 hours at 120° (p_H ca. 5.0).

When thus provided with vitamin B₂ the rats, presumably because they had not exhausted their stores of vitamin B₁, continued to grow. The rats on the caseinogen and yeast extract diet took slightly longer (about 18 days) to become stationary in weight than those on the egg-white

diet (about 15 days). When their weight had been stationary for a week the rats received doses of vitamin B₁-containing substances.

Table I. *Diets employed in the estimation of vitamins B₁ and B₂.*

Diet	Protein g.	Rice starch g.	Arachis oil g.	Salt mixture* g.	Distilled water cc.
P2L	Purified caseinogen 100	300	75	25	500
Used (a) as basal diet devoid of all B vitamins; (b) in vitamin B ₂ tests, with the equivalent of 0.6 g. dry yeast of Peters's antineuritic concentrate (YE) daily as source of vitamin B ₁ .					
FL	"Light white casein" (B.D.H.) 100	300	75	25	500
Used in vitamin B ₁ tests, with a daily dose equivalent to 0.5 g. dry yeast of an autoclaved yeast extract (F5A) daily.					
EL	Egg-white (Protein 100 800 { Water 700	300	75	25	50
Used in vitamin B ₁ tests, the vitamin B ₂ being present in the egg-white. All diets were cooked, after mixing, in a steamer for 3-5 hours according to quantity. 0.05-0.1 g. of cod-liver oil was given daily to all rats to provide vitamins A and D.					

* No. 185. McCollum, Simmonds and Pitz [1917].

Vitamin B₂. The diet used was P2L (Table I). At the end of one week of total depletion of B vitamins the rats received doses of the vitamin B₂-containing substances and at the same time 0.1 cc. (equivalent to 0.6 g. dry yeast) daily of Peters's antineuritic concentrate from yeast (YE) [Peters, 1924; Kinnorsley and Peters, 1925].

A control rat from each litter received vitamin B₁ only, to ensure that no appreciable growth took place.

In addition to these experiments, in which the criterion for vitamin B₂ was growth promotion, the curative effect on the dermatitis which developed in the vitamin B₂-deficient rats was also observed. Comparable results were however difficult to obtain owing to the variability in the time of onset of symptoms and in their nature and degree of severity.

In the growth tests rats were observed for 5 weeks after receiving their doses of vitamin B₁- or B₂-containing substances and the vitamin content was then estimated by comparing the amounts needed to stimulate an increase in weight of 50-60 g. in 5 weeks with the amount of yeast required.

MATERIALS TESTED.

The following vegetables were tested for their vitamin B₁ and vitamin B₂ content respectively.

1. Watercress.
2. Lettuce, cabbage variety. These leaves were pale green.
3. Cabbage. Dark green leaves of different varieties were used without distinction. The etiolated inner leaves of the white-hearted varieties were tested separately.
4. Spinach (*Spinacea oleracea*).
5. Onion (Spanish variety). The onion bulb is morphologically comparable with the other vegetables tested.

A few experiments were carried out with fresh samples, but for the most part dried samples were used. These were prepared by plunging the leaves,

previously washed and freed of stalks and midribs, in boiling water for a minute (this by disorganising the cells accelerates the drying), spreading on sheets of paper and drying at 37° for 48 hours. They were then ground to a fine powder in a coffee mill. The onion was scalded, minced and dried for about 96 hours at 37° on metal sheets before grinding.

The fresh watercress, lettuce and cabbage yielded 9 % of dried material, the spinach 11 % and the onion 6 %.

1. The vitamin B₁ content of the vegetables.

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

Table II. *Vitamin B₁ content of vegetables as demonstrated by the weight increase of rats during 5 weeks on a basal diet, complete, except for vitamin B₁, together with a daily dose of the vegetable to be tested.*

Four rats received each dose, two on the egg-white diet (EL) and two on the caseinogen and autoclaved fraction from yeast diet (FL.F5A).

Material tested	Dry weight g.	Increase in weight in 5 weeks (averages g.)		
		Diet EL	Diet FL.F5A	Average
Watercress	0.3	70	93	81
	0.5	95	77	86
Lettuce	0.15	9	8	8
	0.3	49	58	53
	0.5	85	99	92
Cabbage, etiolated leaves	0.3	32	54	43
	0.5	66	72	69
Cabbage, dark green leaves	0.3	38	29	33
	0.9* (10 %)	—	—	85
Spinach	0.5	63	53	58
Onion	0.5	(9)	35	35
	1.0	57	59	58

* The dark green cabbage was in this case the sole source of the vitamin B complex.

Watercress and lettuce. Among the vegetables tested watercress was the richest source of vitamin B₁, an amount small enough to limit growth was not found, 0.3 g. being the least tested. Lettuce came next, rats receiving 0.3 g. gained 53 g., in 5 weeks.

Cabbage. Etiolated cabbage leaves came next, rats receiving 0.3 g. gained 43 g., those receiving 0.5 g. gained 69 g. Dark green cabbage leaves appeared slightly inferior to the white ones.

Spinach. Rats receiving 0.5 g. of spinach gained 58 g. in weight in 5 weeks, this being slightly less than was obtained with the same dose of etiolated cabbage leaves.

Onion. Onion was found to be a poorer source of vitamin B₁ than the foregoing vegetables. The rats receiving 1 g. gained 56 g., the same as those receiving 0.5 g. of spinach.

These results are summarised in Table IV A where the amounts needed to give 50–60 g. increase in weight in 5 weeks are compared.

The above leafy vegetables may be reckoned rich sources of vitamin B₁, being, when dry weights are compared, about one quarter to one-half as rich as yeast, wheat germ or ox-liver [Chick and Copping, 1930], nearly twice as good as egg-yolk [Chick and Roscoe, 1929], and two to three times better than ox-muscle [Chick and Copping, 1930]. When a comparison of their wet weights is made, they take a lower place owing to their high water content.

As far as any comparison can be made between the different standards adopted, the vitamin B₁ values here recorded are of the same order of magnitude as those of Osborne and Mendel [1919, 1, 2; 1920] and of Sherman [1926]. These workers found however that spinach was richer in the antineuritic vitamin than cabbage.

2. *A third factor affecting the growth of rats.*

Chick and Roscoe [1929] showed that in some cases better growth was obtained with rats receiving a caseinogen and autoclaved yeast diet than with those receiving an egg-white diet, although the dose of vitamin B₁ was the same. A further discussion of this problem is given in the paper by Chick and Copping [1930] and an explanation is put forward, that some dietary factor other than vitamin B₂, provisionally called "factor Y," is present in autoclaved yeast but absent from egg-white.

If in the above experiments the growth be compared when the two different diets were used, it will be seen that no difference in the results was apparent when the rats received doses of watercress, dark green cabbage leaves or spinach. A difference so slight as to be problematic was observed with doses of 0.3 g. and 0.5 g. of lettuce and etiolated cabbage leaves and a more marked difference with doses of 0.5 g. of onion, which disappeared when the dose was doubled.

This result is interpreted to indicate that the unknown dietary factor Y is provided in adequate amounts in watercress, dark green cabbage leaves and spinach, present in rather less amounts in lettuce and etiolated cabbage leaves and in much smaller amounts in onion.

3. *The vitamin B₂ content of the vegetables.*

The results of these experiments are given in Table III. The amounts of the vegetables are expressed as the dry weights eaten daily.

Watercress and spinach. These had similar vitamin B₂ contents, rats eating 0.4 g. in each case increased in weight by the same amount, 55 g. in 5 weeks. Watercress may be slightly better than spinach as rats receiving 0.2 g. also gained 53 g.

Lettuce. This appears to be slightly poorer in vitamin B₂ than watercress and spinach; rats receiving 0.6 g. gained 55 g.

Cabbage. The dark green leaves of the cabbage had the same vitamin B₂

content as watercress or spinach, rats eating 0.4 g. gained 58 g. in weight. The etiolated leaves, however, were poorer even than lettuce, with doses of 0.8 g., 65 g. increase in weight was observed, but only 36 g. with doses of 0.4 g.

Table III. *Vitamin B₂ content of vegetables and yeast as demonstrated by the weight increase of rats during 5 weeks on a diet (P2L.YE) complete except for vitamin B₂, together with a daily dose of the vegetable to be tested.*

Material tested	Dry weight g. daily	No. of rats	Growth in 5 weeks Averages g.
Yeast	0.1	4	50
	0.2	1	75
	0.25	2	85
	0.4	2	110
	10 % $\equiv$ 1.0*	4	110
Watercress	0.2	3	53
	0.3	3	57
	0.4	5	56
Lettuce	0.2	2	44
	0.3	5	46
	0.6	4	57
Cabbage, etiolated leaves	0.2	2	34
	0.3	3	35
	0.4	4	36
	0.8	4	65
Cabbage, dark green leaves	0.2	2	41
	0.4	5	58
	10 % $\equiv$ 0.9*	2	85
Spinach	0.2	3	41
	0.4	5	54
	0.6	4	67
	0.8	4	83
Onion	0.8	2	21
	25 % $\equiv$ 1.6*	4	53

* The foodstuffs were in these cases given as a percentage of the diet.

Onion. This was the poorest source of vitamin B₂. Rats gained only 20 g. in 5 weeks when consuming 0.8 g. daily, or 53 g. with 1.6 g. (given as 25 % of the diet). Larger doses were not tried.

Yeast. In Table III are also given figures for dried brewer's yeast. With daily doses of 0.1 g. the average increase in weight was 50 g. in 5 weeks.

These results are summarised in Table IV B where the amounts needed to provide enough vitamin B₂ for 50–60 g. increase in weight in 5 weeks are compared.

The green vegetables tested were from one-eighth to one-half as rich in vitamin B₂ as dried yeast. They are thus poorer than ox-liver, about equal to milk, steak or egg-yolk and considerably better than the cereals or pulses [Aykroyd and Roscoe, 1929], these comparisons being made on the dry weights. If their wet weights are considered, however, they are inferior even to the cereals, though still equal to milk.

In Table V are shown the amounts of these vegetables needed to cure dermatitis developed in rats on a diet deficient in vitamin B₂. It will be seen that daily doses of 0.3 g. of dry watercress, slightly more than 0.2 g. of spinach,

Table IV. *The vitamin B₁ and B₂ contents of certain vegetables as compared with that of yeast.*

Minimal amounts needed daily to promote an increase in weight of 50–60 g. in 5 weeks in young rats of 40 g. weight.

Material	A As source of vitamin B ₁		B As source of vitamin B ₂	
	Dry weight	Wet weight	Dry weight	Wet weight
	g.	g.	g.	g.
Yeast	0.1?	0.5?	0.1	0.5
Watercress	< 0.3	< 3.75	0.2–0.4	2.5–5.0
Lettuce	0.3	3.75	0.6	7.5
Cabbage, etiolated leaves	0.3–0.5	3.75–6.25	0.4–0.8	5.0–10.0
Cabbage, dark green leaves	> 0.3–0.5	> 3.75–6.25	0.4	5.0
Spinach	0.5	4.5	0.4	3.6
Onion	1.0	16.6	1.6	26.6

0.4 g. of lettuce and 0.5 g. of etiolated cabbage leaves were needed to cure these symptoms. With onion in one case a daily dose of 0.5 g. appeared to cure, but in a more severe case no cure was obtained with 0.8 g. daily.

Table V. *Vitamin B₂ content of vegetables as demonstrated by the curative effect of the vegetables when fed to rats which had developed dermatitis on a diet devoid of vitamin B₂ (P2L.YE).*

Material tested	Dry weight g.	Rat No.	Days on deficient diet	Symptoms	Cure
Watercress	0.2	15070 ♂	42	Eyes closed, fur on face poor. No skin symptoms	Condition became worse
	0.4		49	Eyes very bad, fur very poor	Cured in 3 weeks
	0.3	7005 ♂	140	Inflamed patch on ear. Eyes slightly spectacled	Cured in 3 weeks
Lettuce	0.2	9021 ♂	82	Eyes nearly shut, spectacled. Fur poor	Further symptoms developed
	0.4		98	Skin peeling round eyes and on face, very bad state	Cured slowly
	0.3	15089 ♀	75	Eyes very bad, fur poor	No improvement
Cabbage, white	0.4		82	Nose and face losing hair	Cured in 3 weeks
	0.4	12043 ♂	98	Eyes nearly shut, fur on face poor. No skin symptoms	Eyes cured
	0.3	18097 ♂	63	Skin of one finger on each hand affected. Fur poor	No improvement
	0.5		75	Symptoms as before and losing hair round mouth	Cured in 3 weeks
	0.5				
Spinach	0.2	14061 ♀	123	Eyes spectacled. No skin symptoms	Eyes cured in 3 weeks
	0.2	26157 ♂	105	Sores on abdomen. Fur poor	No cure in 3 weeks
Onion	0.5	15078 ♂	114	Right eye very bad. Coat poor with bald patches, but no inflammation	Eye cured and coat improved but was still not normal
	0.5	24143 ♂	156	Sore patches on head and back. General conditions very bad	General condition became worse. Symptoms the same
	0.8		165	Symptoms as before	Symptoms stationary. General condition very bad. No cure

These figures are only approximate, but it appears that less of the vegetables is needed to cure dermatitis than is needed for good growth. Those that are richest in growth-promoting power have also the strongest curative action.

In addition to the above, an experiment was carried out in which the rats received 10 % lettuce and 10 % dark cabbage, respectively, in the diet as sole source of the vitamin B complex. The growth of these rats for 15 weeks was compared with that of rats receiving 10 % of dry yeast in the diet, and was found to be as good. This would seem to prove that all the factors of the vitamin B complex necessary for the normal growth of the young rat are present in these quantities of lettuce or dark green cabbage leaves.

In order that it might be certain that no portion of the vitamins B_1 and B_2 originally present had been destroyed in the preparation of the dried samples, control rats were fed with the fresh leaves. No appreciable difference was observed either in the vitamin B_1 or B_2 content. The samples were not kept for more than about 2 months, and in this time no deterioration in the content of either vitamin was observed.

The above results suggest that the vitamin B_2 content of the vegetables tested varied with the degree of greenness, being greatest in the darkest green vegetables and least in the colourless onion. It is possible, however, that the figures obtained for lettuce, etiolated cabbage leaves and onion were to some extent lowered by a deficiency of the third hitherto unrecognised factor Y, which appears to be present in insufficient amounts for good growth in these vegetables and also to be present in only small quantities in Peters's anti-neuritic concentrate (see Section 2).

4. *The effect of cooking on the vitamin B_1 and vitamin B_2 content of spinach.*

The spinach was freed of stalks and midribs, placed in a pan with a little water and boiled for 15 minutes. Much juice comes out in this time, so that the shrunken leaves are finally covered by the liquid; this is drained off by pressing by hand in a colander. The only deviations from the domestic method of cooking were that distilled water was used and no seasoning was added.

Vitamins B_1 and B_2 were estimated in the cooked pressed spinach after drying, and in the juice. The results are summarised in Table VI.

With equivalent doses of the dried cooked spinach and of the juice, given as source of vitamin B_1 , the rats gained similar amounts of weight. About half of the vitamin B_1 had presumably passed into the juice. When the cooked spinach and juice were given as source of vitamin B_2 , the rats receiving the juice gained more in weight than those receiving an equivalent amount of the cooked spinach. More than half the vitamin B_2 therefore appeared to have passed into the juice. It is however possible that the vitamin B_2 might be more readily absorbed by the rat from the juice than from the solid.

Table VI. *The effect of cooking on the vitamin B₁ and B₂ content of spinach.*

The estimation was made by comparing the growth-promoting power for young rats of daily doses of the dried uncooked spinach and of the dried cooked spinach and the juice from it.

As source of vitamin B ₁				As source of vitamin B ₂			
Material	Dose	No. of rats	Average increase in weight in 5 weeks g.	Dose	No. of rats	Average increase in weight in 5 weeks g.	
Uncooked	0.5 g. dry*	4	58	0.6 g. dry†	4	67	
Cooked	0.5 g. dry†	3	34	0.6 g. dry‡	4	47	
Juice	2.0 cc.†	2	30	2.4 cc.‡	2	58	
* From 4.5 g. fresh.				† From 5.5 g. fresh.			
				‡ From 6.4 g. fresh.			

From these results it appears that in the domestic cooking of spinach half of both B vitamins is lost in the water.

Previous work on the vitamin B content of cooked vegetables has in the main been devoted to the investigation of the destructive action of heat. Thus McCollum and Davis [1915], Chick and Hume [1917] and Sherman and Grose [1923] among other writers showed that cooking for as much as 4 hours at 100° did not materially affect the water-soluble B vitamins.

Dunham [1921], however, investigated the extractive effect of the cooking water when various vegetables were cooked according to domestic methods. His criterion was the maintenance of weight without growth of young rats, and his results may therefore be taken as vitamin B₁ estimations. He found that the following percentages of the vitamin were lost: potatoes, boiled without the skins 48 %, boiled with skins 32 %, baked 32 %; carrots, sliced and boiled 34 %; cabbage, boiled 26.5 %; canned peas, boiled 8 %.

The cabbage tested by him lost only half as much vitamin B₁ as the spinach here tested. This is probably due to the greater loss of juice from the spinach.

Miller [1920] and Whipple [1920] investigated the loss of vitamin in the cooking of carrots, navy beans, cabbage and onion and found that about 50 % passed into the water. Their method was, however, that of yeast-growth, which has since been shown not to be specific for the water-soluble B vitamins necessary for rat growth.

SUMMARY.

1. Methods for estimation of vitamins B₁ and B₂ using the growth of rats as criterion are described. They contain a few modifications of those previously published from this laboratory.

2. Watercress, lettuce, spinach and cabbage (both the outer dark green leaves and inner etiolated ones), when dry weights are considered, have a content of vitamin B₁ and of vitamin B₂ about one-fourth that of dry brewer's yeast. Their content of vitamin B₁ is lower than that of wheat germ or ox-liver and higher than that of egg-yolk or ox-muscle. The content of vitamin B₂ is lower than that of ox-liver, equal to that of milk, ox-muscle or egg-yolk

and higher than that of the pulses or cereals. In their natural states, however, owing to their high water content, they are relatively poor sources of these vitamins.

3. The onion bulb, composed of colourless leaves, is poorer in both these vitamins than the green leaves, having about one-twelfth to one-sixteenth the value of dry yeast.

4. The distribution of vitamin B₂ appears to bear some relation to the greenness of the leaf, being greater in the dark green leaves than in the paler ones. No such difference is found in the case of vitamin B₁.

5. The vegetables tested were on the whole richer sources of vitamin B₁ than of vitamin B₂.

6. In the cooking of spinach by the domestic process about half of both vitamins B₁ and B₂ is lost in the cooking water and juice.

7. Evidence is put forward in confirmation of the theory, that when egg-white is given as source of vitamin B₂, the lack of a third factor (factor Y) necessary for the normal growth of the rat becomes apparent. This factor is present in autoclaved yeast extracts and in all the vegetables here tested, but the amounts present in lettuce and etiolated cabbage leaves are less than in the dark green leaves tested, and the amount present in the onion bulb is still smaller.

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