XCIV. ON THE COMPOSITE NATURE OF THE WATER-SOLUBLE B VITAMIN¹.

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IN 1915 McCollum and Davis [1915, 1, 2] came to the conclusion that a water-soluble accessory factor was necessary in diet in addition to the fat-soluble vitamin already discovered by them [1913]. This conclusion was based upon the results of a long series of experiments upon young growing rats. McCollum and his colleagues also expressed the opinion that there existed two, and only two, such accessory factors in diet, which were named respectively water-soluble B and fat-soluble A [McCollum and Kennedy, 1916]. The water-soluble accessory substance was found present in milk, egg-yolk and wheat embryo, was soluble in alcohol as well as in water, and withstood prolonged heating at the boiling point of water.

The distribution and properties of water-soluble B, as far as this was investigated, were similar to those of the so-called antineuritic or antiberberi vitamin which had been discovered by Eijkman [1897] and his colleagues as the result of their studies upon induced polyneuritis in birds and their clinical observations upon the etiology of human beriberi. McCollum and Kennedy [1916] concluded that the water-soluble B vitamin was identical with the antineuritic vitamin and that "in the production of polyneuritis in birds by exclusive rice feeding or exclusive feeding of a ration made up of purified foodstuffs, the degeneration of the nerve cells is the specific result of a lack of water-soluble B" [1916, p. 501]. This opinion was widely adopted and emphasised by other authorities; for example by Drummond [1917] and by the Medical Research Council in their Report upon the Present State of Knowledge Concerning Accessory Food Factors [1919, pp. 35, 40].

There were, however, several facts already recorded which were incompatible with this view. Schaumann [1911], Cooper [1912] and Chick and Hume [1917, 2] had pointed out that some materials, mostly extracts and concentrates made from various natural foodstuffs, which were potent to cure or prevent polyneuritis, were incapable of maintaining the weight of the

¹ In this paper the term *antineuritic vitamin* signifies the substance necessary in a diet to prevent polyneuritis in birds and paralysis in rats. The term *vitamin B* (Goldberger's Pellagra-preventive, P-P, factor) is confined to the water-soluble vitamin needed to prevent the pellagra-like disorder in rats with lesions of the skin and alimentary tract. The term *water-soluble B vitamin*, or *vitamins* (the water-soluble B dietary factor of McCollum and his colleagues) signifies a combination of the above two vitamins, each of which is necessary to maintain growth and health.

experimental birds, whereas others had both actions. Cooper [1912] emphasised this fact. Working with several naturally occurring foods, he showed that the respective daily doses which must be added to the birds' diet of polished rice to maintain body weight and to prevent polyneuritis respectively, were different and that there was no regular relation between them.

During the last ten years, evidence has been steadily accumulating which is in opposition to the theory that these two vitamins are identical, notwithstanding the fact that their distribution is so similar in naturally occurring foodstuffs. A comprehensive review of the evidence on this point was given in a paper by Mitchell [1919]. More evidence has, however, been added since that date, among others by Heller [1923], Willaman and Olsen [1923], Emmet and Stockholm [1920], and Levene and Muhlfeld [1923].

The evidence against the identity of water-soluble B with the antineuritic vitamin may be summarised under three different headings:

1. *Distribution in nature.* While this is generally the same, there are important quantitative differences. Some materials, for example pure wheat embryo, are rich sources of antineuritic vitamin [Chick and Hume, 1917, 1, 2] but poor in water-soluble B [Osborne and Mendel, 1919, 2]. In the case of milk, meat, green leaves and roots and tubers the reverse is true [Cooper, 1914; Hopkins, 1912, 1920; Osborne and Mendel, 1922; Gibson and Concepcion, 1916; McCollum and Kennedy, 1916; Osborne and Mendel, 1919, 1, 2; 1920; 1922; Chick and Hume, 1917, 3; Vedder and Clark, 1912; Sugiura and Benedict, 1918; McCollum, Simmonds and Parsons, 1918.]

Randoin and Lecoq [1926] have shown that certain yeasts of different origin, while possessing equal power to maintain growth, vary considerably in their antineuritic properties.

2. *Heat stability.* Foodstuffs containing these vitamins can be heated to 100° for 1–2 hours without appreciable loss; at higher temperatures, e.g. 120°, the antineuritic vitamin has been found much more sensitive than water-soluble B [Chick and Hume, 1917, 2; Sherman and Grose, 1923; Emmet and Luros, 1920].

3. *Solubility and other properties.* Both principles are soluble in water and can be dialysed. Water-soluble vitamin B is less soluble than the antineuritic vitamin in strong alcohol [Funk, 1912; Cooper, 1913; Kinnersley and Peters, 1925; Drummond, 1917], acetone [Abderhalden, 1920; McCollum and Kennedy, 1916; Steenbock, 1917; McCollum and Simmonds, 1918] and benzene [McCollum and Simmonds, 1918; McCollum and Kennedy, 1916]. Both vitamins can be adsorbed upon animal charcoal or fuller's earth, but adsorption appears to be more complete in case of water-soluble B and recovery more difficult after processes involving precipitation [Drummond, 1917].

Further light has been shed upon the problem recently by the investigations of Goldberger and his colleagues upon pellagra. Those workers have been led to abandon their previous theory that the cause of this disorder is to be traced to an inferior biological value of the protein in the diet. In their

attempts to prevent human pellagra they found that while such a valuable protein as caseinogen (up to 90 g. daily) gave disappointing results, liberal daily supplements of butter-milk (1200 g.), fresh meat (200 g.) or 30–50 g. dried brewer's yeast (and less of certain extracts), were able to cure or prevent pellagra in persons receiving diets on which this disease might confidently be expected to develop [Goldberger and Tanner, 1924, 1925; Goldberger, Wheeler, Lillie and Rogers, 1926]. These foods are all rich sources of water-soluble B and after extending the work to a series of observations on young growing rats [Goldberger, Wheeler, Lillie, and Rogers, 1926; Goldberger and Lillie, 1926] the conclusion has been reached that the results can best be explained by supposing that what McCollum and Kennedy described as water-soluble B vitamin consists of two substances. These are (1) a pellagra-preventive, P-P, factor, and (2) an antineuritic vitamin, both constituents being required to maintain growth and health in the young rat, (1) being less soluble in alcohol than (2) and more resistant to heat. The facts on which this theory is based are, shortly, these. When young rats were maintained on a basal diet deprived of the water-soluble B vitamin but including as much as 30–40 % of yeast heated in an autoclave at 15 lb. pressure for $2\frac{1}{2}$ hours, growth was not maintained and death occurred after a period of 8–10 weeks, preceded in some cases by symptoms of paralysis. If, however, the diet was further supplemented by a small daily dose of an alcoholic extract of corn-meal to provide antineuritic vitamin, the animal grew or remained in normal health even if the dose of autoclaved yeast were much diminished. If, on the other hand, the basal diet was supplemented *only with the antineuritic substance*, growth was not maintained, even when a comparatively large dose was administered. No paralysis occurred but, after a variable period following the arrest of growth, a number of such animals developed a series of symptoms which were considered to resemble human pellagra [Goldberger and Lillie, 1926]. The shortest time taken for development was 7 weeks. The symptoms included ophthalmia, loss of fur and characteristic dermatitis on ears, paws and neck. With the inclusion of a small amount, 6 %, of a preparation made with acidified water from autoclaved yeast, these symptoms cleared up and growth was resumed.

The present experiments confirm the work of Goldberger and his colleagues and support the conclusion drawn by those workers and the other observers already referred to, that McCollum's water-soluble B vitamin and Eijkman's antineuritic substance are not identical.

The work arose out of an examination of the alcoholic extract made from the material adsorbed on charcoal in Peters' [1924] process for purification of the antineuritic vitamin contained in brewer's yeast. The extract had been prepared by Sir C. J. Martin in the hope that it might provide a possible standard for comparison in the titration of foodstuffs for content of the water-soluble B vitamin. Prof. Peters had told us that although the material was highly potent for the cure of polyneuritis induced in pigeons on a diet of

polished rice, he had no experience of its capacity to maintain growth in rats on a diet deprived of the water-soluble vitamin B.

This was investigated, and it was found that the extract would not encourage growth. If, however, supplemented with a daily ration of yeast heated at 120° for 5 hours to destroy the antineuritic vitamin, the rats grew normally and health was maintained.

A series of experiments has been carried out in which yeast and wheat embryo have been taken as sources of the two water-soluble B vitamins. Various extracts and products have been prepared from these and used both alone and in combination, to provide supplementary rations for the basal diet deprived of these vitamins. Observation has been made of the different pathological conditions developed in the rats and a quantitative study made of the substances required for prevention and cure.

EXPERIMENTAL.

The following materials have been prepared and investigated.

Yeast. Whole dried yeast. Fresh brewer's yeast was washed by kneading in ice-cold water to a smooth paste, filtering and pressing, the operation being repeated three or four times. The washed and pressed yeast was dried at 37° for 3 days and ground to a powder in a mill.

Autoclaved yeast. The above dried yeast was heated for 5 hours in an autoclave at 120° and then dried again at 37°. It did not alter in weight but the colour changed from light to dark brown.

Yeast extract [Peters' method, 1924]. The material used was prepared from the extract with acidified 50 % alcohol (alcohol 50 : water 50 : concentrated HCl 1, by volume) of the material adsorbed on animal charcoal after the precipitations by lead acetate and acid mercuric sulphate had been carried out. In our preparation the traces of mercury remaining were removed, before adsorption with animal charcoal, by passing SH_2 through the solution. The precipitation was completed and excess of sulphuric acid removed by adding the appropriate amount of BaCO_3 , excess being avoided. The acid alcoholic extract was concentrated at a low temperature under reduced pressure and the residue taken up in water. 1 cc. of the final solution administered was equivalent to about 7 g. of the original washed yeast (dry weight).

Wheat embryo. The wheat embryo was a specially pure specimen containing very little bran; it was dried at 37°.

Autoclaved wheat embryo was prepared by autoclaving for 5 hours at 120° and then drying at 37°.

Two extracts were prepared from the wheat embryo.

Absolute alcohol extract. Wheat germ dried at 37° was shaken at room temperature for periods of 5 hours with each of two changes of absolute alcohol. The filtrates were taken almost to dryness under reduced pressure at approximately 40° and the residue shaken up with ether and acidified water. The water-soluble fraction, after being dried and redissolved in water, was tested. It is called the *absolute alcohol extract*.

85 % alcohol extract. The wheat germ used in the previous extraction was re-extracted by shaking at room temperature for periods of 5 hours with each of two changes of 85 % alcohol (85 cc. absolute alcohol made up to 100 cc. with distilled water). The combined filtrates were reduced to dryness as before and taken up in an amount of water equivalent to the original weight of wheat germ. This is the *85 % alcohol extract*.

The following are the details of the experiments. Black and white or albino rats were used and were placed upon the experimental diet when 3-4 weeks old and weighing 40-50 g. They were kept in cages with screened

wire bottoms of $\frac{1}{2}$ -inch mesh to prevent contact with the faeces and probable ingestion [see Steenbock, Sell and Nelson, 1923]. The diet was given in a semi-liquid condition in small pots.

The basal diet deprived of water-soluble B vitamins, hereafter called Diet K, consisted of:

Purified caseinogen (specially extracted with ether and alcohol)	21 parts
Rice starch	63 „
Cotton-seed oil	11 „
Salt mixture ¹	5 „

From 0.05 to 0.1 g. cod-liver oil was given to each rat daily according to the size.

1. *Effect on rats of diets lacking in the antineuritic or both water-soluble B vitamins.*

When young rats, 40–50 g. in weight, are placed upon the basal diet K, very slight growth may take place, but the appetite fails and the body weight gradually declines. After 3–4 weeks the body weight is usually about 35 g., the animals are emaciated and present a feeble and miserable appearance. If untreated, they die, usually without any obvious symptoms other than those of inanition.

Table I. *Effect of yeast and wheat embryo—dried, autoclaved or given as extracts—on the cure and prevention of the collapse (so-called “neuritis”) occurring in young rats after 3–4 weeks on diet K deprived of water-soluble B vitamins.*

Exp.	Material	Daily dose required for prevention	Daily dose required for cure
A	Yeast, dried	—	0.05 g.
	„ autoclaved	None with 0.2 g.	No cure or alleviation with 0.2 g.
	„ extract (Peters)	—	0.1 cc.*
B	Wheat embryo, dried	—	0.1 g.
	„ autoclaved	None with 0.4 g.	No cure or alleviation with 0.2 g.
	„ extract with absolute alcohol	—	No cure or alleviation with equiv. of 1.0 g. wheat germ
	„ extract with 85 % alcohol	0.2 cc.†	0.3 cc.

* Equivalent to 0.7 g. yeast.

† Equivalent to 0.2 g. embryo.

Symptoms of paralysis (see below) were only rarely observed in animals receiving this treatment. Such was also the experience of Drummond, who noted paralytic symptoms in 3 only out of a large number of young rats [Drummond, 1918] on a diet deprived of water-soluble vitamins. Schaumann [1910] observed nervous symptoms in adult rats after 3–4 weeks on a diet of horseflesh heated with alkali to 120° for 1 hour, or of rye bread similarly treated. Hofmeister [1922] has given a detailed study of the paralysis in rats caused by lack of water-soluble vitamins in the diet. He found that the

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

condition could be best studied when developed in a more chronic form and was of the opinion that when this happened it was probable that the deficiency was not quite complete, otherwise death occurred swiftly and suddenly. In the present experiments with young rats death from inanition usually occurred before there had been time for the development of paralysis. In one series of experiments however (litter 541, Table IV) 5 out of 8 rats in one litter developed symptoms of paralysis within 33 days after receiving the experimental diet. The onset of the paralysis occurred as described by Hofmeister. It was marked by loss of appetite and great restlessness; there was also a progressive loss of co-ordination and of power in the hind legs. The rats swayed from side to side in walking and sometimes would fall down on one side. Later on, the animals maintained a position with the back hunched up and the head bent forward; they became unable to stand upright unless holding on with the forepaws and frequently fell over upon the head. Two animals (rats 29 and 31, Table IV) in this condition were cured with 0.1 cc. of Peters' yeast extract, and three died.

In the majority of cases definite symptoms of paralysis were not observed, and it was at the time of general collapse, occurring after 3-4 weeks upon the experimental diet K, that the effect of curative substances was tested (see Table I). Administration of Peters' antineuritic extract (0.1 cc. daily, equal to 0.7 g. yeast) or the (85 %) alcoholic extract of wheat embryo (0.3 cc. daily, equivalent to 0.3 g. embryo) restored the animal to some degree of health, and weight was maintained for a considerable period, although there was no significant increase. These extracts also cured paralytic symptoms promptly in cases where these were observed (rats 29 and 31, Table IV).

The extract from wheat embryo made with absolute alcohol was without effect in relieving the symptoms of paralysis (see rats 28, 32, 33, Table IV). The antineuritic substance in wheat embryo thus appears to be insoluble or only sparingly soluble in absolute alcohol; that present in the extract prepared from dried yeast by the method of Kinnersley and Peters [1925] is stated however to be soluble in absolute alcohol.

If dried yeast or wheat embryo were given in sufficient amount (yeast 0.05 g.; wheat embryo 0.1 g.) the animals were cured, were restored to health and also began to grow. In the autoclaved yeast or autoclaved wheat germ, on the other hand, the antineuritic properties had been destroyed by the strong heating. When these were administered no cures were effected in rats showing paralysis (rat 28, Table IV) or in those suffering from inanition, and the animals died in the same way as those which were untreated.

2. Development of "pellagra" in rats due to lack of the second of the water-soluble vitamins.

Young rats which received the basal diet K supplemented with alcoholic extracts made from yeast or wheat embryo did not grow but showed no signs of collapse at 3-4 weeks. After about 6-8 weeks had elapsed they sometimes

died without showing any special external symptoms, but in other cases they gradually developed the condition described by Goldberger and his colleagues (see above) and considered by these workers to resemble human pellagra. The body weight was usually maintained (rat 68, Curve III, Fig. 2), but the animals were in poor condition, they were thin and active, with rough coat and with the

Table II. *Effect of yeast and wheat embryo—dried, autoclaved or given as extracts—on the cure and prevention of “pellagra” occurring in young rats after 6–8 weeks upon diet K, deprived of water-soluble B vitamins, but supplemented by the antineuritic vitamin.*

Exp.	Material	Daily dose required for prevention, g.	Daily dose required for cure, g.
A	Yeast, dried	0.1 + (0.05 –)	—
	„ autoclaved	0.2 + (0.1 –)	0.2
	„ extract (Peters)	None with 0.3 cc.*	—
B	Wheat embryo, dried	0.2	—
	„ autoclaved	0.4	0.4 did not cure
	„ extract with 85 % alcohol	0.8 cc.† delayed onset	—
	* Equivalent to 2.1 g. yeast.		† Equivalent to 0.8 g. embryo.

In cases where no doses are given marked with the – sign, smaller doses were not tried.

urine often slightly blood stained. Loss of hair took place, and red inflamed patches of skin appeared on the nose and on the backs of the fore feet, which later became oedematous. There was a marked conjunctivitis and in some cases the ears also became red and thickened. The inflamed areas spread and often involved the hind legs and whole areas round the nose and mouth; sometimes the chest became bare and inflamed. The animals survived 2–3 weeks or longer in this condition before death occurred. On *post mortem* examination there were no definite macroscopic signs except a very unhealthy condition of the whole alimentary tract, especially in the small intestine which showed signs of inflammation with atrophy of the mucous membrane and often contained blood-stained mucous.

The condition was also developed in rats which had been saved from collapse on basal diet K by administration of the alcoholic extracts of yeast (rat 12, Curve II, Fig. 1) or of wheat embryo (rat 52, Curve I, Fig. 2). The time elapsing was usually 3–4 weeks after the previous collapse and 6–8 weeks from the beginning of the experiment.

This “pellagrous” condition was cured slowly but surely by addition to the diet of a small daily ration (0.2 g. and upwards) of autoclaved yeast (see Table II A and rat 12, Curve II, Fig. 1), and at the same time growth was restored. Several days, however, usually elapsed before there was a definite improvement. The skin lesions gradually healed, desquamation took place on chest and paws and round nose and mouth; thickened, cheesy layers peeled from the ears, leaving them thin and papery. In about 2 weeks the skin was healed and hair was beginning to grow.

Fig. 1. Influence of the water-soluble vitamins contained in dried yeast upon the growth of young rats receiving the basal diet K.

Curve I. Rat 59 ♂ on basal diet without addition. Death at point N due to lack of antineuritic vitamin.

II. Rat 12 ♂ on basal diet without addition for 3½ weeks. Collapse prevented at point N by addition of 0.1–0.3 cc. yeast extract daily (Peters' method). After 6 weeks symptoms of "pellagra" at point P, cured and growth restored by administration of 0.2 g. autoclaved yeast daily.

III. Rat 61 ♀ on basal diet without addition for 3 weeks. Collapse prevented and growth restored at point N by addition of 0.2 cc. yeast extract and 0.3 g. autoclaved yeast daily.

IV. Rat 62 ♀ on basal diet + 0.2 g. autoclaved yeast daily for 3½ weeks. Collapse prevented and growth restored at point N by addition of 0.1 cc. yeast extract daily.

V. Rat 55 ♂ on basal diet + 0.2 g. daily of dried yeast. Normal growth.

Rats 55, 59, 61, 62 were of one litter.

N = Collapse or death due to lack of antineuritic vitamin.

P = Development of skin lesions ("pellagra") due to lack of vitamin B (Goldberger's "pellagra-preventive" factor).

- = Basal diet only.
- x ----- = Basal diet with daily addition of antineuritic extract of yeast or of wheat germ.
- = Basal diet with daily addition of autoclaved yeast or wheat germ.
- x ——— = Basal diet with daily addition of antineuritic extract as well as autoclaved yeast or wheat germ.
- = Basal diet with daily addition of whole dried yeast or wheat germ.

3. Experiments on growth (see Table III and Figs. 1 and 2).

Yeast. All symptoms were prevented and some degree of growth was maintained with a very small daily supplement of whole dried yeast (0.1 g.). Some degree of growth was shown also with 0.1 g. of wheat embryo, while normal growth (weekly increase of 10 g. and upwards) was obtained with

0.2–0.4 g. daily of dried yeast or 1.0 g. of wheat embryo (Table III, Exps. A and C).

When the rats received doses of autoclaved yeast up to 0.2 g. daily (rat 62, Curve IV, Fig. 1) there was no evidence of growth, but if supplemented by 0.1 cc. daily of the yeast extract, steady growth took place, although it was subnormal (7 g. a week, Table III B). A normal increase in body weight took place when the rats received 0.4 g. autoclaved yeast together with 0.1 cc. of the yeast extract. Since neither of these materials given alone in similar amount could maintain growth, but could respectively relieve the “pellagrous” and neuritic symptoms, the conclusion is obvious that whole yeast contains both principles, that both are necessary for growth and health, that the antineuritic vitamin is destroyed in the autoclaved yeast and that vitamin B (Goldberger’s P-P factor) is absent from this particular yeast extract.

Drummond [1918] found that extracts made from yeast by methods involving precipitation processes had lost the power of promoting growth in rats, whereas a number of other observers, including Peters and ourselves, find similar extracts to be potent antineuritic substances.

Table III. *Effect on growth of young rats of vitamin B, or the “pellagra-preventive” (P-P) factor, in presence of adequate antineuritic vitamin.*

Ex- per- iment	Material	Daily dose, g.	Given as preventive		Given as cure for collapse due to lack of antineuritic vitamin or of vitamin B	
			No. of animals	Average weekly increase in body wt. g.	No. of animals	Average weekly increase in body wt. g.
A	Yeast, dried	0.05	—	—	1	1
		0.1	—	—	1	3
		*0.2	1	11	3	9
		0.3	—	—	1	7.5
		0.4	1	23	—	—
B	Yeast, autoclaved (antineuritic vitamin supplied as Peters’ yeast extract)	0.1	—	—	1	4
		0.2	1	7	3	5
		*0.3	—	—	2	13
		0.4	1	10	1	10
C	Wheat embryo, dried	0.1	—	—	1	3
		0.2	1	2	3	3
		0.4	—	—	2	1.5
		0.6	1	6	1	6
		*1.0	2	13	—	—
		1.5	1	12	—	—
D	Wheat embryo, autoclaved (anti- neuritic vitamin supplied as 85% alcoholic extract of wheat em- bryo or as Peters’ yeast extract)	0.2	—	—	1	0
		0.4	1	2	2	4
		0.6	—	—	1	4
		0.8	—	—	2	4
		1.0	1	5	—	—
E	Wheat embryo, dried + 0.3 g. autoclaved yeast	0.2	1	15	—	—
		0.4	2	15	—	—
		0.6	1	15	—	—

* Dose required for normal growth.

Fig. 2. Influence of the water-soluble vitamins contained in wheat embryo upon the growth of young rats receiving the basal diet K.

- Curve I. Rat 52 ♀ on basal diet without addition for 3½ weeks. Collapse prevented at point N by addition of 0.7 cc. wheat embryo extract (85 % alcohol) daily. After 9 weeks symptoms of "pellagra" at point P.
- II. Rat 30 ♀ on basal diet without addition for 4 weeks. Collapse prevented and growth restored at point N by addition of 0.2 g. wheat embryo daily.
- III. Rat 68 ♂ on basal diet + 0.2 cc. wheat embryo extract (85 % alcohol) daily. Symptoms of "pellagra" at point P followed by death 2 weeks later due to lack of vitamin B (Goldberger's P-P).
- IV. Rat 67 ♂ on basal diet + 0.4 g. autoclaved wheat embryo daily. Death after 3½ weeks due to lack of antineuritic vitamin.
- V. Rat 84 ♀ on basal diet + 1.0 g. wheat embryo daily. Normal growth.

Table IV. Result of adding wheat embryo and preparations of wheat embryo to diet K of litter 541:—8 young rats 27 days old, of weight 41–44 g., of which 5 rats developed symptoms of paresis after 4–5 weeks on the basal diet.

Rat no.	Initial wt. g.	Paresis		Body wt. at onset, g.	Whole embryo g.	Daily supplements of wheat embryo and preparations given		Time on basal diet only, before supplement, days	Result
		Present	Day of onset			Auto-claved embryo g.	Absolute alcohol extract cc.		
26 ♂	41	0	—	—	—	—	—	—	Died on 26th day
32 ♀	42	+	32	32	—	—	0.2–0.4*	30	" 35th "
33 ♀	42	+	33	31	—	—	0.2–0.4	30	" 36th "
28 ♂	43	+	28	30	—	0.2	0.2–0.4	28†	" 29th "
29 ♂	42	+	33	36	—	0.2 later 0.4	0.2–0.4	30	Cured with 0.1 cc. yeast extract, given from the 36th day
31 ♀	42	+	33	36	—	"	0.2–0.4	30	" " "
27 ♂	42	0	—	—	0.1	—	—	21	Symptoms prevented
30 ♀	44	0	—	—	0.1	—	—	30	" " "

* Equivalent to 3–6 g. of the original wheat embryo.

† Autoclaved wheat embryo given from the 23rd day.

Wheat embryo. The evidence of the existence of the two water-soluble B vitamins in wheat embryo is not quite as dramatic as with yeast. The explanation seems to be that the wheat embryo, if not mixed with other portions of the grain, is comparatively poor in the P-P constituent, while a rich source of the antineuritic factor. Osborne and Mendel [1919, 2], in growth tests with rats, found that commercial wheat embryo, *i.e.* a sample admixed with bran, was a better source of water-soluble B vitamin than a very pure sample of the embryo picked off the seed by hand in the laboratory. The sample of embryo used in the present case was specially pure and contained only a very small proportion of bran or other products. With their commercial wheat embryo Osborne and Mendel obtained good growth with doses less than 0.2 g. daily; with the present specimen 1.0 g. daily was required (see Table III C).

That the P-P factor is present in wheat embryo in very small amount compared with its content of antineuritic vitamin is also evident from the following facts.

(1) When the whole wheat embryo was supplemented with a small daily dose, 0.3 g., of autoclaved yeast, normal growth (an increase of 15 g. weekly) was obtained with a daily dose of only 0.2 g. daily of the embryo (Table III E).

(2) Using the autoclaved embryo, supplemented with antineuritic vitamin from another source, only subnormal growth showing 5 g. increase in weight per week was obtained with a daily dose of 1 g. (Table III D).

(3) The 85 % alcoholic extract of the embryo, which should contain both constituents, if present in the original material, was found to be a good source of the antineuritic vitamin (see Table I B) but to have only a slight effect in preventing the onset of "pellagra" (Table II B).

DISCUSSION.

From the work of others and of ourselves described above, it is clear that the water-soluble B dietary factor of McCollum and his colleagues is not one substance but two at least. McCollum and Kennedy [1916] were evidently mistaken when they adopted the simplest interpretation of the facts at their disposal and pronounced water-soluble B to be identical with the antineuritic substance concerned with the prevention and cure of polyneuritis in birds.

As there are two vitamins two names are required. We would propose to retain the name *antineuritic vitamin* for the antineuritic substance, discovered by Eijkman in 1897 which cured and prevented polyneuritis in birds fed upon a diet of polished rice and was later found to be necessary to prevent marasmus and paresis in mammals.

We suggest that the name *vitamin B* be provisionally confined to the second water-soluble vitamin contained in the dietary factor B discovered by McCollum and Davis in 1915. The substance provisionally called the pellagra-preventive or P-P dietary factor by Goldberger and his colleagues [1926] appears to be vitamin B in this restricted sense. Its presence in a diet is necessary to maintain health in young rats and to prevent decline and death

accompanied by severe skin lesions. Goldberger and his colleagues consider that this condition is the analogue of human pellagra and suggest that deficiency of this vitamin is the cause of the human disease.

As both these water-soluble vitamins are necessary for continued growth and health, it follows that both are present in substances hitherto found to satisfy the requirements of McCollum's water-soluble B, as originally described.

These two vitamins, while both soluble in water, appear (1) to have different solubilities in strong alcohol, benzene and acetone, (2) to possess differing capacities for adsorption on various finely divided solids and (3) to have a markedly different resistance to heat. These differences have made it possible to separate them from one another as they occur in yeast and in wheat embryo, and have provided a method for the study of one apart from the other. On further investigation it may prove that their distribution in nature is by no means as similar as it now appears to be.

In making the above suggestions with regard to nomenclature, we fully realise the incomplete state of our present knowledge concerning the water-soluble B vitamins and the possibility that McCollum's water-soluble B may eventually be shown to contain more constituents than the two dealt with in this paper. The principles responsible for the effects described in experiments of Hartwell [1922, 1, 2], Drummond and Reader [1926], Boas [1927], may or may not prove to be identical with one or other of these two vitamins already differentiated.

SUMMARY.

1. Experiments are described with young growing rats which confirm the views held by many investigators, and recently emphasised by Goldberger and his colleagues, indicating that the water-soluble B vitamin of McCollum and Kennedy contains at least two constituents.

(i) The *antineuritic* (or antiberiberi) vitamin which prevents or cures the collapse, accompanied in some cases with symptoms of paralysis, and leading to death, which occurs in young rats of 40–50 g. weight after 3–4 weeks upon a diet devoid of water-soluble B vitamins. It is identical with the antineuritic substance, discovered by Eijkman in 1897, necessary to prevent polyneuritis in birds fed upon a diet of polished rice.

(ii) The *vitamin B sensu strictu*, identical with the pellagra-preventive or P-P factor of Goldberger and his colleagues. This vitamin can prevent or cure the marasmus accompanied by severe skin lesions, which develops in young rats after 6–8 weeks upon the deficient diet. The effect of its absence can only be demonstrated in cases where the animal is provided with the antineuritic factor in order to prevent the collapse which otherwise would occur at an earlier date.

All known substances containing the water-soluble B vitamin, as defined by McCollum and his colleagues, contain both constituents, since both are necessary for maintenance of growth and health.

2. It is suggested that the term *vitamin B* should be confined to this second water-soluble vitamin (Goldberger's P-P factor), distinct from the antineuritic vitamin, but commonly accompanying it in natural foodstuffs.

3. Yeast is a rich source of both water-soluble B vitamins; pure wheat embryo, while rich in the antineuritic vitamin, was found to be comparatively poor in vitamin B (Goldberger's P-P factor).

4. Vitamin B (P-P factor), as was shown by Goldberger and his colleagues, is much more thermostable than the antineuritic vitamin. A preparation practically free from the antineuritic vitamin can be obtained by heating yeast in an autoclave at 120° C. for 4-5 hours.

5. The strongly antineuritic material extracted from the charcoal in Peters' method of purifying the antineuritic vitamin in yeast was found to be devoid of vitamin B (Goldberger's P-P factor).

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