LVI. A METHOD FOR THE ASSAY OF THE ANTINEURITIC VITAMIN B₁, IN WHICH THE GROWTH OF YOUNG RATS IS USED AS A CRITERION.

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THE antineuritic vitamin (vitamin B₁ in the nomenclature provisionally adopted by the Biochemical Society) was discovered in 1897 by Eijkman [1897] and was recognised by its ability to prevent and cure polyneuritis which developed in birds fed upon a restricted diet. The general distribution of this vitamin in natural foodstuffs was worked out by Eijkman and his colleague Grijns [1901] and quantitative assay was later attempted by Cooper [1913, 1914] and by Chick and Hume [1917, 1, 2] by comparison of the minimal doses required to prevent (or cure) the polyneuritis of pigeons fed upon a diet of polished rice.

The antineuritic B₁ vitamin was formerly believed to be identical with, and is now known to be one constituent of, the complex "water-soluble B" discovered by McCollum and Davis [1915, 1] and found by them to be necessary in a diet to ensure normal growth in young rats.

It is now certain [Goldberger *et al.*, 1926; Chick and Roscoe, 1927] that the antineuritic vitamin B₁, equally with the second identified constituent of "water-soluble B," viz. the heat-stable, antidermatitis, (?) antipellagra, vitamin B₂, is necessary for normal growth of young mammals. Young rats just weaned are so sensitive to absence of vitamin B₁ that they seldom survive more than 3-4 weeks even if vitamin B₂ is provided, death usually taking place from inanition without symptoms of paralysis. If, however, the antineuritic vitamin is administered before the condition of the animal is too far advanced recovery takes place and normal growth is established. In rats the suddenness of death and the inconstancy of the symptoms following vitamin B₁ deficiency make it impossible to assay this vitamin by any method based upon cure or recovery in the acute stage. But if foodstuffs containing this vitamin are administered as soon as the animal's weight has become stationary the increase in body weight which follows can be used as a criterion for estimating the content of vitamin B₁ in the material fed.

The present method for assay of this vitamin has been developed along the lines of that previously described for the assay of vitamin B₂ [Chick and Roscoe, 1928] and used by Aykroyd and Roscoe [1929] in their study of the distribution of this vitamin. In that method the provision of vitamin B₁ unaccompanied by vitamin B₂ is conveniently arranged by administering

Peters's antineuritic concentrate prepared from yeast. But in the analogous method for assay of vitamin B₁ the provision of vitamin B₂ unaccompanied by the former is not so easy. We have used washed brewery yeast, autoclaved at 120° for 5 hours, which retains about one-half the vitamin B₂ potency of the original yeast and is at the same time practically devoid of vitamin B₁. Sometimes, however, traces of vitamin B₁ appear to survive this treatment, the result, doubtless, depending on the amount of vitamin B₁ contained in the original yeast. Another drawback to the use of autoclaved yeast is its tendency to cause diarrhoea. Further heating to destroy all traces of vitamin B₁ is not advisable, for the amount of the deleterious substances would thereby be increased, and as more vitamin B₂ would also be destroyed, larger doses would be required.

The occurrence of diarrhoea complicates experiments with B vitamins for two reasons. If severe, the animal naturally suffers apart from any dietetic deficiency; if slight, the loose stools passed do not fall cleanly through the meshed wire floor of the cage [Chick and Roscoe, 1928, p. 796] and more opportunity is afforded for faeces-eating, which is a common habit among rats deprived of B vitamins and tends to mitigate the effects of the deficiency [Steenbock, Sell and Nelson, 1923; Kennedy and Palmer, 1928].

A more convenient, but, for reasons stated below, less satisfactory, source of vitamin B₂ free from vitamin B₁ was found in the fresh white of hen's eggs, a surprising fact, since the yolk contains both factors in abundance. One can only conclude that the yolk membrane is impervious to the passage of vitamin B₁.

A basal diet (E.L. diet) was prepared, in which the purified caseinogen of our usual "-B" diet (P₂L diet) was replaced by egg-white (see Table I). The relative amounts of dry constituents were the same as in the P₂L diet, the protein amounting to 20 % in either case. In preparing the diet the egg-white is separated from fresh eggs and coagulated by heating in a double saucepan containing boiling water, put through a mincing machine and then thoroughly mixed with the dry constituents of the diet, the small extra amount of water being worked in afterwards. The prepared diet is steamed for 3 hours at 100° in order to cook the starch thoroughly and prevent the risk of "refection" [Fridericia *et al.* 1928; Roscoe, 1928].

Table I. *Basal diets used in the study of the (antineuritic) vitamin B₁.*

E.L. (egg-white) diet. (g.)		P ₂ L (caseinogen) diet. (g.)	
Egg-white 800.	Protein 100	Purified caseinogen	100
	Water 700	Rice starch	300
Rice starch	300	Cotton seed oil	75
Cotton seed oil	75	Salt mixture (McCollum's 185)	25
Salt mixture (McCollum's 185)	25	Distilled water	500
Distilled water	50		
	1250		1000

0.4 g. autoclaved yeast daily was given to rats on diet P₂L to provide vitamin B₂. Cod-liver oil, to supply vitamins A and D, was administered to all rats separately by hand, 3 to 5 drops (0.05 g. to 0.1 g.) according to the size.

Young rats (40–50 g. weight), receiving the egg-white diet shortly after weaning, usually continued to grow for 2–3 weeks, after which the weight declined for lack of vitamin B₁. Death occurred if this was unchecked, but if Peters's antineuritic concentrate were given (0.1 cc. daily, equivalent to 0.6 g. dry yeast), the animals recovered and began to grow at the normal rate. In many cases they continued to grow normally for several weeks but a check was usually observed sooner or later before maturity was reached.

With young rats on the P₂L (caseinogen) diet supplemented with 0.4 g. autoclaved yeast daily to provide vitamin B₂, the preliminary period of growth generally lasted a week or two longer, owing possibly to traces of undestroyed vitamin B₁, which had survived the strong heating. In some

Fig. 1. Failure to grow of young rats from Litter 1184 on diets deprived of the antineuritic vitamin B₁, but containing egg-white (diet E.L.) and autoclaved yeast, respectively, as sources of vitamin B₂.

Rats 414 and 410 restored by equal doses of the same vitamin B₁-containing yeast extract; rat 407, by Peters's antineuritic concentrate.

instances the degree of growth displayed later during recovery, during the test of the vitamin B₁-containing material, was also greater than in rats receiving the egg-white diet. But in many cases concordant results were obtained when two rats of the same sex and litter, after suitable preparation on the two respective diets, received the same dose of vitamin B₁ (see Table II). This is illustrated by the weight curves of the four rats shown in Fig. 1. Two rats, 407 and 410, received E.L. diet and began to decline in weight after 2 weeks. After 4 weeks 407 was *in extremis* but was revived by a daily dose of Peters's antineuritic concentrate. Of the two rats on diet P₂L with a daily addition of 0.4 g. autoclaved yeast, 414 also grew for 2 weeks and then rapidly lost weight, but rat 412 was more resistant and attained the weight of 73 g. on this diet and after 6 weeks showed no sign of vitamin B₁ deficiency, although the body weight (68 g.) was then slowly falling.

Rats 414 and 410, after 2½ weeks and 3 weeks on their respective diets deprived of vitamin B₁, were treated with the same dose of a yeast fraction

containing this vitamin. The degree of growth induced was the same in both cases (see also Table II, Exp. 1).

In Table II are collected the results of a series of tests in which similar doses of vitamin B₁-containing materials were administered to pairs of rats prepared respectively on the two different basal diets. In series (a) the results are fairly concordant, in series (b) the rats receiving autoclaved yeast grew somewhat better than those on the egg-white diet.

It is hoped to make a survey of the antineuritic (vitamin B₁) content of some of the commoner foodstuffs for comparison (a) with that already made, using prevention and cure of polyneuritis of birds as a criterion, and (b) with the distribution of vitamin B₂ already published by Aykroyd and Roscoe [1929]. If the unit of vitamin B₁ be defined as the dose required to restore normal (11–14 g. weekly) growth to a young rat, of which the growth has failed on a basal diet deficient only in vitamin B₁ and containing excess of vitamin B₂, the assay of a foodstuff consists in determining the minimal dose necessary for this degree of restoration.

Table II. *Comparison of (antineuritic) vitamin B₁ assay using basal diets containing egg-white (diet E.L.) and autoclaved yeast (diet P.Y.) respectively as sources of vitamin B₂.*

Series	Exp.	Material	Daily dose	Litter	Rat	Body wt, g.	Diet	Weekly increase in wt, g.	Av.
(a)	1	Yeast Fraction X ₅	Equiv. to 0.12 g. dry yeast	1184	410 ♀	50	E.L.	13, 16, 12	14
				"	414 ♀	51	P.Y.	17, 12, 16	15
	2	" XI ₂ C	" 0.25 "	1173	397 ♀	59	E.L.	9, 14	11.5
				1184	406 ♂	61	P.Y.	11, 14	12.5
	3	" XII C	" 0.25 "	1184	409 ♀	45	E.L.	21, 13, 14	16
				1173	396 ♀	39	P.Y.	16, 13, 7, 10	11.5
	4	" XII Ca	" 1.0 "	1118	373 ♀	49	E.L.	22	22
				1117	361 ♂	43	P.Y.	23, 34	28
	5	" XII ₅	" 0.12 "	1118	368 ♂	51	E.L.	20, 17, 11, 15, 14	15
				1117	360 ♂	40	P.Y.	18, 17, 7, 17, 11	14
(b)	6	" XII ₅ C	" 0.25 "	1291	462 ♂	46	E.L.	15, 18, 20, 21	18.5
				1298	470 ♂	41	"	17, 13, 18, 15	16
				1291	459 ♂	61	P.Y.	26, 21, 18, 13	19.5
				1298	468 ♂	54	"	28, 10, 18, 15	18
	7	Dried egg yolk	0.5 g. = 1.9 fresh	1291	464 ♀	40	E.L.	2, 2, 1	2
				"	461 ♀	51	P.Y.	7, 3, -1	3
		"	1.0 g. = 3.9 fresh	1291	465 ♀	38	E.L.	16, 18, 13, 8	14
				1298	466 ♂	72	P.Y.	26, 19, 12, 9	18.5

The basal diet containing egg-white as source of vitamin B₂ would be in every way the more convenient of the two diets described, were it not that this diet, even when supplemented with adequate vitamin B₁, as Peters's antineuritic concentrate, does not maintain growth of rats to maturity. That egg-white contains in abundance the antidermatitis vitamin B₂ is shown by the many satisfactory cures of the "pellagrous" condition which have been obtained with comparatively small doses [Aykroyd and Roscoe, 1929]. It would seem that some third factor in the vitamin B complex, other than vitamins B₁ and B₂, is present in autoclaved yeast, and lacking in egg-white.

The heat stability of this hypothetical third substance is an argument against its identity with those suggested by the work of Williams and Waterman [1927, 1928] and of Reader [1928].

The matter is being investigated further. So far our observations do not permit a definite conclusion to be drawn upon the extent to which the absence of this hypothetical third factor may affect the validity of the test for vitamin B₁ described in this paper. In a few instances the growth of rats has become subnormal in the first few weeks after weaning, but it has usually remained unaffected until at least 6–10 weeks after weaning (about 9–13 weeks from birth), by which time the animal has attained a body weight of 100–130 g. No symptoms of ill health have been found in rats observed for three to four months after growth has become sub-normal. Nevertheless, pending the results of further investigation it seems safer, in the assay of vitamin B₁, to use autoclaved yeast as the source of vitamin B₂ in the diet rather than egg-white.

SUMMARY.

1. Methods are described for assay of the antineuritic vitamin B₁, using growth of young rats as a criterion and basal diets in which the more heat-stable vitamin B₂ is supplied by fresh egg-white or by yeast autoclaved at 120° for 5 hours.

2. Fresh egg-white, while rich in vitamin B₂, is devoid of vitamin B₁. With autoclaved yeast there is a risk that traces of vitamin B₁ which have escaped destruction in the autoclave may be present. Nevertheless autoclaved yeast is to be preferred to egg-white for the reason given in 3, below.

3. Diets otherwise complete, which contain egg-white and Peters's antineuritic concentrate as sources of vitamins B₂ and B₁ respectively, are frequently found unable to maintain normal growth of rats beyond a few weeks after weaning. After this period growth is subnormal. It is suggested that a third hypothetical dietary factor may be present in the vitamin B complex, which factor is heat-stable, present in autoclaved yeast and lacking in egg-white.

4. If the unit of vitamin B₁ be defined as the amount which will restore normal growth (weekly increase in weight of about 10–14 g.) in young rats whose growth has failed on a basal diet deficient only in this vitamin, assay of a foodstuff for content of vitamin B₁ would consist in determination of the minimum dose necessary to restore this degree of growth.

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