CXLIV. NOTE ON THE INTERNATIONAL STANDARD FOR THE ANTINEURITIC VITAMIN B₁.

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(Received June 27th, 1932.)

THE vitamin B₁ preparation adopted as International Standard by the League of Nations Conference on Vitamin Standards, held in London in June 1931, is an adsorbate on fuller's earth obtained by Seidell's method [1916] from an extract of rice polishings, the extract being made with water containing 20 % alcohol and acidified with sulphuric acid to p_H 4·5 [League of Nations Health Organisation, 1931]. 3 kg. of the fuller's earth represent the antineuritic vitamin B₁ from 100 kg. of rice polishings. This adsorbate, which is prepared in the Medical Laboratory at Batavia (Java) and used in that district for the treatment of beriberi, formed the starting-point for the preparation of the crystalline vitamin B₁ product isolated by Jansen and Donath [1927].

Before its adoption as a vitamin B₁ standard, this "acid clay" adsorption product was investigated by several members of the Conference to ascertain its degree of suitability for use as a standard. At the request of the Accessory Food Factors Committee (of the Medical Research Council and Lister Institute) quantitative tests using different methods were made in various laboratories in this country and abroad; in these tests the adsorbate was used as a standard of comparison in the estimation of the potency of other vitamin B₁-rich substances—dried yeast, wheat embryo, *etc.* Standard specimens of these materials were distributed to the various laboratories and in the course of tests carried out at the Lister Institute the following results were obtained which seem worthy of record, as being possibly of general interest and of use to other workers in this field.

In the method of vitamin B₁ estimation employed in the following tests, the criterion adopted was the growth of young rats [Chick and Roscoe, 1929]. A basal diet was used (caseinogen 100; rice starch 300; salt mixture McCollum No. 185, 25; arachis oil 75; water 550; with cod-liver oil 50–100 mg. daily administered separately), to which the antidermatitis vitamin B₂ was added as a small daily ration of an autoclaved (4 hours, 120°, at p_H 5·0) watery yeast extract given in amount equivalent to 0·5 g. of the yeast (dry wt.) from which the extract was prepared [see Roscoe, 1930]. The standard degree of "normal" growth adopted was an average weekly increase in body weight of 10–14 g. While the tests were in progress there was an exceptionally cold period, lasting about two weeks, during which it was impossible to keep the animal room at the usual temperature and the growth of the rats under observation was checked. In calculating the average weekly increments in growth, those corresponding to this period have been omitted.

The vitamin B₁ standard adsorption product is a fine white powder easy to administer and readily taken. The minimal daily dose required to support an average weekly increase of 10–14 g. was found to be about 10 mg. (see Table I) and for convenience in preparing the doses the material was diluted ten-fold with pure dextrin and thus administered. The results of the series of tests summarised in Table I show the standard to be about 6 and 12 times as

Table I. *Comparative (antineuritic) vitamin B₁ value of (a) the standard adsorption product from rice polishings, (b) dried yeast, (c) wheat embryo, and (d) Peters's antineuritic concentrate from yeast.*

(Vitamin B₂ supplied as autoclaved yeast extract, daily dose $\equiv$ 0.5 g. yeast (dry wt.).)

Material	Daily dose g.	No. of rats used	Av. weekly increment in weight during 5 weeks' observation g.	Av. weekly increment in growth during	
				1st week g.	5th week g.
(a) International Standard ...	0.025	1	16.2		
	0.012	6	14.3	15.0	13.5
	0.009	4	11.5	15.0	8.2
	0.006	4	7.7	8.5	6.5
	0.010	2	11.4	19.0	9.0
	0.0075	2	9.7	13.0	4.0
	0.010	2	10.6	14.5	11.0
	0.0075	2	8.1	14.0	5.0
	0.10	3	17.5	17.5	12.0
	0.075	5	14.6	17.2	11.0
(b) Dried yeast	0.05	5	10.9	9.7	11.5
	0.20	4	16.4	17.0	10.0
	0.15	4	15.2	17.7	9.2
(c) Wheat embryo ...	0.10	6	10.3	13.5	7.2
	cc.				
	0.10	4	17.2	18.0	17.0
(d) Peters's antineuritic concentrate from yeast	0.075	2	17.4	21.0	15.0
	(0.1 cc. $\equiv$ 0.6 g. yeast dry weight)	3	15.4	17.7	11.0

potent respectively as the samples of dry yeast and wheat embryo tested. The International unit of vitamin B₁ is defined as the "antineuritic activity of 10 milligrams of the international standard adsorption product" [Report, 1931, p. 76]. The sample of dried yeast examined would therefore contain 16–18 International vitamin B₁ units per g. and the sample of wheat embryo 8 units per g.

It has frequently been found that when young rats are fed on a simplified "complete" diet containing the minimum amounts of B-vitamins for "normal" growth, the rate of increase in weight may show a falling off after a few weeks; in the estimation of vitamin B₁ this may happen towards the end of the five weeks' period of the test (see Table I, column 5). In the present experiment this occurred to some extent when vitamin B₁ was given as dried yeast, but to a greater extent when wheat embryo or the standard adsorption material from rice polishings was used and more especially after the latter material had been stored for a year (Table I).

It is noteworthy that in most investigations of the B-vitamins, those factors have been studied as they occur in yeast and yeast products. Failure in continued growth on a "complete" experimental diet has frequently been observed when the yeast products have been purified or when the constituents of the B-vitamin complex have been derived from other natural substances. To explain the relative failure when vitamin B₂ was supplied by a preparation from egg-white rather than by an autoclaved yeast extract Chick and Copping [1930] and Roscoe [1930] postulated the existence of factor Y, an unknown constituent of the yeast vitamin complex abundantly present in yeast and some other foods and lacking in egg-white. In the present tests, however, factor Y would be provided in the autoclaved yeast extract given as source of vitamin B₂. The relatively heat-labile vitamin B₄, found by Reader [1929, 1930, 1, 2] to be necessary for rat growth, would be supplied in the acid clay ration, seeing that the crystalline vitamin B₁ product finally obtained from this material by Jansen and Donath's method was found by Jansen *et al.* [1930] to be an unusually rich source of vitamin B₄.

It is probable that our knowledge of the B-vitamin complex might be extended and some of the discrepancies characteristic of the subject removed by a further study of these vitamins as they occur in natural materials other than yeast. In the meantime the existing evidence suggests the presence in yeast of other essential dietary factors in addition to those of the vitamin B complex which have been already recognised.

Stability of the standard vitamin B₁ adsorption product. This material was reported from the Medical Laboratory, Batavia, to possess great stability. In the present investigation tests made after storage for 10–12 months, both in a refrigerator and in an incubator room maintained at 37°, showed little, if any, deterioration to have taken place in the original vitamin B₁ potency (Table I).

Content of vitamin B₂. A few growth tests were made on young rats receiving diets in which the standard adsorption product was given as source of vitamin B₂, and vitamin B₁ was provided as Peters's purified concentrate from yeast (method of Chick and Roscoe [1928]). Only an insignificant degree of growth (3–4 g. weekly) was obtained with daily doses of 0.05 and 0.1 g.; with 0.25 g. daily the average weekly increment in weight was only 6.5 g. The vitamin B₁ content of the standard material is therefore more than 25 times, and probably at least 50 times, greater than its content of vitamin B₂ and the amount of the latter contained in the small daily dose of 10 mg. required to supply vitamin B₁ might be regarded as negligible.

This standard vitamin B₁ could therefore be used as a source of vitamin B₁, sufficiently free from vitamin B₂, in diets destined for work on the latter vitamin and would provide a much more convenient and economical material for the purpose than Peters's vitamin B₁ concentrate from yeast, which is only obtained after an elaborate and lengthy process of preparation.

SUMMARY.

1. The preparation adopted as International Standard for the antineuritic vitamin B₁ (adsorption product on fuller's earth from an acid, watery extract of rice polishings) has been found exceedingly potent (daily rat dose about 10 mg.), convenient to handle and easy to administer.

2. It possesses a satisfactory degree of stability when preserved for a year either at room temperature, at 37° or in a refrigerator.

3. Its content of vitamin B₂ was found to be negligible in comparison with its vitamin B₁ potency. This material should therefore serve as a useful source of vitamin B₁ in diets designed for the study of vitamin B₂.

In conclusion our best thanks are due to Prof. B. C. P. Jansen for the supply of the standard vitamin B₁ adsorption product with which the above tests were made.

REFERENCES.

- Chick and Copping (1930). *Biochem. J.* **24**, 1764.
—— and Roscoe (1928). *Biochem. J.* **22**, 790.
—— — (1929). *Biochem. J.* **23**, 498.
Jansen and Donath (1927). *Mededeelinger Dienst. Volksgezondheid Ned.-Indië*, **1**, 1.
—— Kinnersley, Peters and Reader (1930). *Chem. Ind.* **49**, 990.
League of Nations Health Organisation (1931). Report of the Permanent Commission of Biological Standardisation, p. 75. (Geneva.)
Reader (1929). *Biochem. J.* **23**, 689.
—— (1930, 1). *Biochem. J.* **24**, 77.
—— (1930, 2). *Biochem. J.* **24**, 1827.
Roscoe (1930). *Biochem. J.* **24**, 1754.
Seidell (1916). *U.S. Public Health Reports*, **31**, 364.