A METHOD OF VITAMIN ASSAY AND ITS APPLICATION IN A STUDY OF THE VITAMIN B AND G CONTENT OF MUNG BEANS AND THE GRAIN SORGHUMS

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Following the demonstration of the existence of the two factors, vitamins B and G in the water soluble vitamin B complex, there was begun a study of the method of assay of the anti-neuritic factor, vitamin B. An examination of the literature disclosed a lack of uniformity in the composition of the basal vitamin B complex-free diets which had been employed by the various investigators. These diets differed especially in their carbohydrate and fat content. The possibility suggested itself that the results of assays carried out with different basal diets might not be comparable. A preliminary study was therefore made to determine to what extent the type of carbohydrate employed in the basal diet affects the results of an assay of vitamin B.

In the procedure employed in the assay of vitamin B albino rats 28 days old and weighing from 40 to 50 grams were given a basal diet devoid of vitamins B and G until cessation of growth indicated a depletion of these vitamins. Vitamin G was then supplied by 0.5 gm. autoclaved yeast daily; vitamin B was administered as an alcoholic extract of rice polishings. Litter mates were given different quantities of this extract in order to determine the minimum amount necessary to enable the animals to make an average daily gain of 1.5 to 2.0 gm. over a period of 21 days. Control animals were continued on the B-free diet alone or supplemented with vitamin G. The basal diet consisted of carbohydrate, 58 per cent; casein (acid-washed) 18; crisco, 15; salt mixture, 3.5; agar-agar, 2; cod-liver oil, 3.5. The carbohydrates used ricestarch, cornstarch, dextrinized cornstarch, sucrose, and a mixture of sucrose 15 per cent and dextrin 43 per cent. It was found that the minimum amount of the vitamin B extract required to produce a gain of 1.5 gm. to 2.0 gm. daily for three weeks was influenced by the carbohydrate employed in the basal ration, as is shown in Chart I.

Of the carbohydrates used, ricestarch proved most satisfactory. The superiority of ricestarch was not due to traces of the vitamin in the carbohydrate since animals which received the ricestarch diet were depleted of their stored vitamin B as rapidly as were the animals which received the other carbohydrates. Control animals of the ricestarch diet did not survive longer than did similar control animals receiving the other basal diets. The diets containing sucrose either alone or with dextrin were especially unfavorable to growth. Animals on these diets showed loss of hair after two weeks and their general appearance indicated an especially low nutritive condition.

When the basal B-free diets contained 3.5, 18.5 and 33.5 per cent fat (Crisco), the same minimum amount of the vitamin B extract was required to produce the desired gain in weight. The basal B-free diet selected to be used in the assay of vitamin B contained ricestarch as the source of carbohydrate and 15 per cent fat.

Graph I shows the growth response of rats to increasing amounts of vitamin B and is a characteristic curve for vitamin B. The number of animals used to establish each point is indicated along the curve. Burns*, following Trevans theory, uses a similar but not identical curve

*J. A. Burns, Physiological Reviews, 10, 1, 146 (1930).

in the biological assay of drugs. Since a unit amount of vitamin B has been defined as the amount which will produce a gain in weight of 32 to 42 gms. in 3 weeks, we may construct what Trevan calls the characteristic for vitamin B in rats. A perpendicular through the curve where the ordinate reaches 32 will cut the abscissa at what may now be called one unit; half-way between this point and the axis will be 0.5 unit, and similarly 1.5, 2.0 units may be marked off. The same curve with units designated on the abscissa is given in Graph II. If the method of assay suggested by Burns for drugs is applicable to the assay of vitamin B, it should be possible to assay a given source of vitamin B by feeding just one amount of the substance instead of several different amounts. The only restriction to the method is that the growth response must fall on the straight line portion of the curve. From the gain in weight induced by a given amount of the substance, one can read directly from the characteristic vitamin B curve the number of units contained in this quantity of the substance and can calculate the amount representing a unit of vitamin B. The obvious advantage of such a method is the smaller number of animals required to assay a given substance.

To test the applicability of this method to the assay of vitamin B, mung beans, kafir, and darso were used as sources of vitamin B and curves were established showing the response in growth to increasing amounts of these substances. These three curves are shown on Graph II. On each of the curves the amount of supplement containing one unit of vitamin B has been determined by a perpendicular drawn through the curve at the point where the ordinate reaches 32 and cutting the abscissa at a point designated as one unit. The units of vitamin B are indicated beneath each graph.

The following table presents a comparison of the vitamin B content of the three supplements determined by use of the individual curves with the unit values obtained by the use of the characteristic vitamin B curve. From the growth response of animals to 0.75 and 1.0 gm. of each of the supplements the unit vitamin B content of each may be read from the characteristic vitamin B curve by means of perpendiculars drawn to the curve where the ordinate reaches the observed growth response. The points at which the perpendiculars cut the abscissa give the number of units of vitamin B contained in a known amount of the supplement.

TABLE I.

The Vitamin B Content of Mung Beans, Kafir and Darso as Determined by Individual Curves and Calculated from the Characteristic Vitamin B Curve.

Supplement	Amount	Gain in wt. in 21 days	Units of Vitamin B		Amount of Supplement containing one unit vitamin B	
			Individual curve	Characteristic curve	Individual curve	Characteristic curve
	gm.	gm.			gm.	gm.
Mung Bean	0.75	37	1.3	1.25	0.6	0.6
	1.0	44	1.7	1.60		
Kafir	0.75	35	1.1	1.15	0.66	0.66
	1.0	43	1.5	1.56		
Darso	0.75	33	1.2	1.10	0.63	0.68
	1.0	38	1.6	1.30		

From the close agreement of these results it may be concluded that the vitamin B content of a food may be determined from the characteristic

vitamin B curve if the growth response of rats to a single amount of the food is known, provided the response falls on the straight line portion of the curve.

Assays of the vitamin G content of mung beans, darso and kafir were made. The grain sorghums were found to be almost devoid of vitamin G. Animals receiving 3.5 gm. of these daily as supplements to the basal G-free diet were unable to gain 3.2 gms. in 21 days. Mung beans proved to be a better source of vitamin G; 2.0 gm. daily suplied sufficient vitamin G to permit an average daily gain of 1.5 gm. over a period of 21 days.

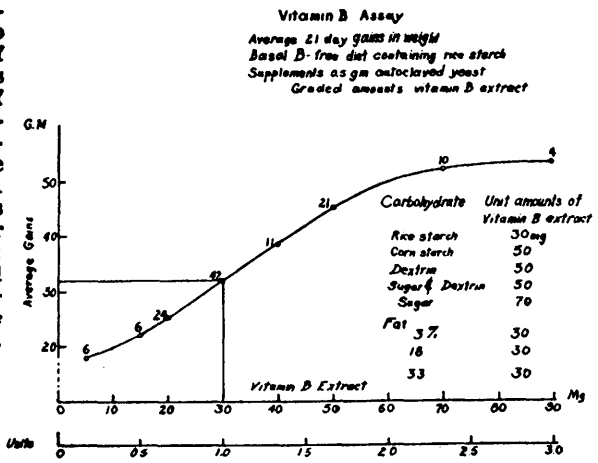

Figure 1. The Growth Response of Rats to Increasing amounts of Vitamin B Extract. A Characteristic Curve for Vitamin B.

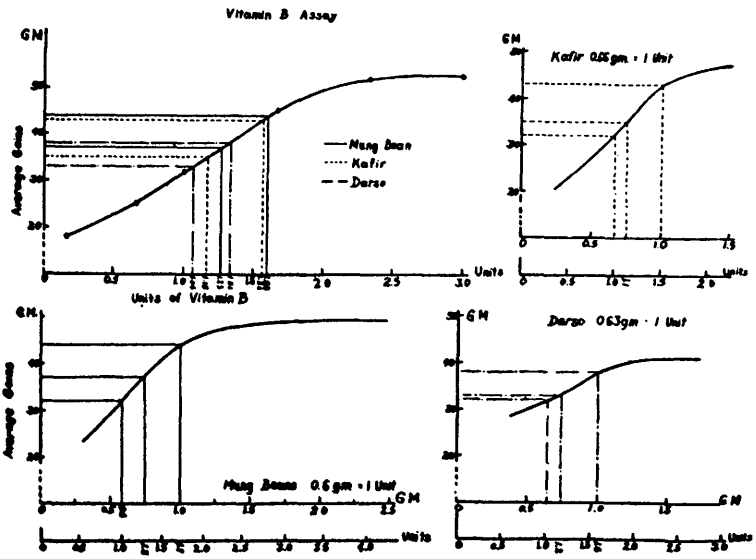

Figure 2. An Assay of the Vitamin B Content of Mung Beans, Kafir and Darso