[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

A QUANTITATIVE STUDY OF THE DETERMINATION OF THE ANTINEURITIC VITAMIN B[1]

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With the generally accepted differentiation of the former vitamin B complex into the antineuritic vitamin B (B_1) and the more heat-stable vitamin G (B_2), there arose the need for quantitative measurements of these separate vitamin potencies, both for studies of food values and for the more exact investigation of problems relating to the distribution of these vitamins in nature and their concentration in the laboratory.

We here summarize briefly the results of our study of such determination of vitamin B in the present sense. The method described is based upon that formerly used for analogous measurements of undifferentiated vitamin B values, vitamin G being now supplied by including "standardized" autoclaved yeast in the basal vitamin B-free diet.

A bakers' yeast, of rather low initial vitamin B (B_1) content, was the material chosen as source of vitamin G.

In our first series of experiments, the yeast was simply heated at fifteen pounds' pressure under steam in open petri dishes in the autoclave (100 g. of air-dry yeast in each dish of 13.5 cm. diameter), the air having first been displaced from the autoclave chamber by means of steam. The test animals receiving this autoclaved yeast gained in weight for from one to two weeks, but survived scarcely longer than the negative controls. The results were practically the same whether the heating under steam pressure was continued for two, four or six hours.

In another series of experiments, the yeast was autoclaved in the same manner, with and without the addition of enough tenth-molar potassium hydroxide to make a smooth paste (approximately 1.3 cc. of 0.10 *M* alkali per gram of air-dry yeast). After the autoclaving, and before the subsequent drying of the yeast, this added alkali was neutralized by the addition of an equivalent amount of hydrochloric acid. This alkalinization of the yeast before autoclaving, suggested by the work of Hassan and Drummond,[2] did not seem to cause any significant difference in the response of the experimental animals to the particular yeast fed in these experiments. Hence it appears that for the particular yeast here used, any of the above autoclave treatments was adequate to destroy all but immeasurably small amounts of the vitamin B present. Williams, Waterman and Gurin,[3] however, have shown that no such experimental find-

[1] Published as Contribution No. 668 from the Department of Chemistry of Columbia University.

[2] A. Hassan and J. C. Drummond, *Biochem. J.*, **21**, 653 (1927).

[3] R. R. Williams, R. E. Waterman and S. Gurin, *J. Biol. Chem.*, **83**, 321 (1929).

ings for any one yeast can safely be assumed to hold true for dried yeasts generally; each laboratory should test the adequacy of its mode of autoclaving for the yeast which it uses.

That the difference between our experience and that of Williams, Waterman and Gurin is due to the difference in the original vitamin B values, the acidities, and possibly also the buffer values of the yeasts employed, has been adequately demonstrated in the subsequent work of Bisbey in this Laboratory.[4] The yeast used here probably was more easily freed from vitamin B (B_1) than the average. Doubtless also the autoclave treatment caused a material diminution of its vitamin G (B_2) content; but at the liberal level of feeding here used, all of our autoclaved yeasts supplied ample amounts of vitamin G for the purposes of our experiments.

Hence each laboratory should very carefully "standardize" its own autoclaved yeast, both as to completeness of destruction of vitamin B and adequacy of vitamin G value of the resulting product. It is plainly advantageous that the yeast selected for this purpose should be of high original vitamin G value and of low original vitamin B value and acidity.

In the experiments here described we have used with satisfactory results, a basal diet (Diet 513) containing: autoclaved yeast (prepared as explained above), 15; casein (freed from vitamins B and G by repeated extraction with 60% alcohol), 18; Osborne and Mendel[5] salt mixture, 4; cod liver oil, 1 (or 2); butterfat, 9 (or 8); starch, 53%.

Normal young rats have been placed upon this diet when four weeks of age and have usually ceased to increase in weight (indicating depletion of bodily surplus of vitamin B) in about two weeks. The interposition of this depletion period before the period of experimental feeding does not appreciably affect the uniformity of the final results in the case of the animals of our highly inbred colony of long-known nutritional history;[6] but it will doubtless tend to greater uniformity of results as between different laboratories, and it naturally affects the number of grams of actual increase of weight of the test animals during the early part of the period of experimental feeding. Hence, there is double reason for the general use of such a depletion period, though in a single series of experiments in a given laboratory it may add nothing to the precision of the comparative values obtained.

Our animals have usually weighed about 65 to 70 g. at the end of the depletion period.

A representative member of each litter is then continued on the basal diet only, to serve as a ("negative") control, while the others receive graded allowances of the food (or other material) which is being tested

[4] B. Bisbey, Dissertation, Columbia University, 1930.

[5] T. B. Osborne and L. B. Mendel, *J. Biol. Chem.*, **37**, 572 (1919).

[6] H. C. Sherman and E. H. MacArthur, *ibid.*, **74**, 107 (1927).

for its vitamin B value. If the material thus tested is already known to be of considerable vitamin B potency, the animals receiving the higher allowances will serve as "positive controls" for the litter as a whole; otherwise, one member of each litter may be made such a control by feeding an adequate amount of any suitable material known to contain vitamin B. The cages are, of course, provided with raised bottoms of wide-mesh wire screen.

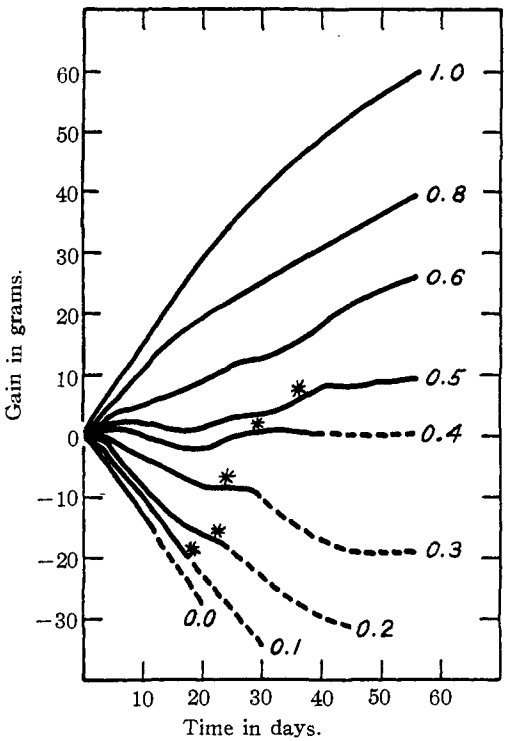

Fig. 1.—Average growth curves of rats receiving different amounts of vitamin B (B_1). The lowest curve shows the result of feeding the basal diet (Diet 513) only; the others, the results of feeding daily, except Sundays, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 and 1.0 gram, respectively, of ground whole wheat as source of vitamin B. Twelve "standard" depleted rats were fed at each level. Broken lines indicate the continuing record of the survivors after one or more had died.

* Indicates the point, for each of five levels of feeding, at which, on the average, distinct polyneuritis appeared. It will be noted that the negative control animals, receiving no vitamin B whatever, usually die before the development of typical polyneuritis; also that polyneuritis is prevented, at least in a majority of cases, by such level of feeding of vitamin B as results in a gain of 3 grams per week, the rate of gain in body weight recommended as a basis for quantitative comparisons and as indicating the intake of one "unit" of vitamin B per day.

The accompanying graph (Fig. 1) shows the average results obtained (upon twelve animals in each case) in a series which included negative controls and eight levels of feeding of vitamin B in the relative proportions of 1, 2, 3, 4, 5, 6, 8 and 10, respectively.

It is believed that these curves may be taken as an approximately correct quantitative expression of the relation between the relative levels of vitamin B feeding and the resulting weight curves of test animals under such systematically controlled conditions as are here indicated. It must be emphasized that a merely routine repetition of experiments of a "standard" description is not sufficient to ensure a truly quantitative result, even with large numbers of animals; large numbers of animals are needed to offset individual variability; but it

is also important that each laboratory attempting quantitative work shall also establish experimentally the adequacy of its own standardization of materials and methods. It is hoped that the results here briefly summarized will serve to assist other workers in the establishment of satisfactorily controlled conditions, and to indicate the quantitative character of the relationships which may be expected from weight curves which are averages of the data obtained by the feeding of such numbers of "standardized" and well-matched "litter-control" experimental animals as are here described.

Throughout the eight weeks of the experimental period shown in Fig. 1, it appears, both from the planning, control and internal evidence of the experiments, and from three years' subsequent experience of this laboratory, that the antineuritic vitamin B (B_1) was the sole growth-limiting factor. We recognize, however, that there may be other vitamins essential to the growth of the rat and not exclusively furnished by our basal diet. In such case the smoothness of the curves shown in Fig. 1 would be attributable in part to the presence of such new or unknown factors in the body of the experimental animal or in the material fed as source of vitamin B, or both It may happen that the bodily store of some unknown factor (with or without the assistance of minute amounts contained in the material under test) will suffice for a part but not the whole of an experimental period as long as eight weeks. If this is the case, there will tend to develop a flattening or possibly even a "break" in the growth curve at the point at which the unknown substance and not vitamin B becomes the growth-limiting factor. In general, the longer the experimental period, the greater the chance that such a vitiating condition may arise. Hence there may be an actual gain in accuracy by shortening the experimental period to four or five weeks. To secure results of the highest quantitative value, it is well to weigh the test animals frequently in the early part of the experimental period so as to establish the exact form of the weight curve in its early stages, and then compute the rate of gain from a four-weeks' section of the weight curve which is rising steadily throughout.

Since increasing the allowance of the vitamin fed naturally does not usually increase the rate of gain in *exactly arithmetical* proportion, quantitative comparisons are greatly facilitated by the adoption of a conventional basis of comparison or "unit" of vitamin B value. This, while conventional, need not be wholly arbitrary. It may be formulated in direct relation to the experimental operation, and to the performance of such experiment at a level of feeding which is advantageous from the combined points of view of accuracy and delicacy. At the low levels of feeding represented by the lower curves in Fig. 1, the response to a given increment of the allowance of vitamin is relatively large; but the individual variations are relatively great, largely because the animals are apt to become distinctly ill. The asterisks on the curves (Fig. 1)

show the points at which, on the average, distinct symptoms of polyneuritis developed. At the levels of vitamin B feeding represented by the higher curves, polyneuritis was prevented and the individual differences were not so great; but also there was a lessened response in growth to a given small increment of vitamin B intake, so that the method while gaining in accuracy was losing in delicacy.

A rate of gain of 3 g. per week affords such a combination of accuracy and delicacy as to be a good basis for quantitative comparisons, and it has the added advantage of being already familiar as the "standard" rate of gain in the analogous determinations of vitamin A values.

We therefore recommend that in order to facilitate quantitative comparisons: a "unit" of vitamin B be considered to be that amount which, when fed as a daily allowance to a standard test animal (rat), under such conditions as have been indicated above, will suffice to support three grams per week of gain in weight during an experimental period of not less than four nor more than eight weeks.

Separate summary and conclusions are here purposely omitted because this paper as a whole is an attempt at concise summary of experimental data too numerous to be printed in detail; and because, as already explained, each quantitative investigator must still establish experimentally his own conclusions as to certain points comprised within the general plan and principles outlined above.

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MIXED BENZOINS. VII. MAXIMAL CATALYTIC REDUCTION

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Several papers have been published on the catalytic reduction of benzoins and related compounds.[1,2,3] While attempting to prepare certain reduction products of some benzoins the authors were unable to obtain end-points for the intermediate stages of reductions. It was therefore decided to reduce the benzoins to the maximum extent. Seven mixed benzoins and benzoin itself were so reduced. From benzoin,[3] *o*-chlorobenzanisoin[4] and *o*-chlorobenzveratroin,[4] α,β-dicyclohexylethane[5] and

[1] Buck and Jenkins, THIS JOURNAL, **51**, 2163 (1929).

[2] Jenkins, Buck and Bigelow, *ibid.*, **52**, 4495 (1930).

[3] Kariyone, *J. Pharm. Soc. Japan*, No. 515, 1 (1925).

[4] Buck and Ide, THIS JOURNAL, **52**, 4107 (1930).

[5] Obtained in other ways by Freundler, *Compt. rend.*, **142**, 343 (1906); *Bull. soc. chim.*, [3] **35**, 541, 549 (1906); Sabatier and Murat, *Compt. rend.*, **154**, 1771 (1912).