TABLE II

ANTIPYRETIC EFFECT OF $CH_3CONHC_6H_4NHCOOR$

Starved rabbits... 2 cc. Typhoid antigen (5 bil/a)
Urethans..... 0.20 g. per kg. body weight
Amidopyrin..... .15 g. per kg. body weight
Acetanilide..... .10 g. per kg. body weight

Urethan Alkyl group (R)	Change in temperature at the end of 3 hours, °F.	5 hours, °F.
Methyl	−0.7	−1.1
Ethyl	−1.0	−1.2
n-Propyl	−1.4	−2.1
Isopropyl	+1.1	−0.2
n-Butyl	+1.2	+ .7
Isobutyl	+0.7	+ .2
Sec.-butyl	+2.2	+1.3
Sec.-amyl	−0.5	−0.9
n-Hexyl	+ .5	−1.1
n-Heptyl	+ .1	−1.5
Amidopyrin	−3.8	−3.7
Acetanilide	−2.4	−0.4
Nothing (received antigen only)	No change (average of five)	−0.1 to −0.3 (average of five)

Summary

A series of *p*-acetylaminophenylurethans was prepared and they were tested for their antipyretic action. The compounds of low molecular weight and high molecular weight exhibited some activity but none of the compounds was as active as amidopyrin or acetanilide. No hypnotic activity was observed in any case.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

QUANTITATIVE DETERMINATION OF VITAMIN G(B_2)[1]

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Our purpose in the work here described[2] has been to develop a method whereby the responses in the weight curves of properly standardized experimental animals may become means of measuring the relative vitamin G (B_2) values of foods or of concentrates obtained in research work.

The factor here designated as vitamin G or B_2 may or may not be identical with that to which the term pellagra-preventive has been applied;

[1] Published as Contribution No. 667 from the Department of Chemistry of Columbia University.

[2] This paper is a brief summary of the work described in the privately printed dissertation submitted by Anne Bourquin in partial fulfilment of the requirements for the degree of Ph.D., Columbia University, 1929, together with the results of subsequent experience in the use of the method in the Columbia laboratories.

but in any case the substance with which we are here concerned is an important factor in normal nutrition.[3]

Shortage of this factor causes retardation or cessation of growth and if the deprivation is sufficiently complete, a gradual loss of weight begins after the body has become sufficiently depleted of any surplus store which it may previously have acquired.

Inasmuch as the quantitative measurements and comparisons with which we are here concerned are based entirely upon the weight curves of the experimental animals, we need not now consider any other symptoms of the G-avitaminosis.

The general plan is to add to a basal diet adequate in other respects but free from the "vitamin B complex," a strong alcohol extract of ground whole wheat (or other material) which supplies an abundance of vitamin B (B_1) with so little vitamin G that the latter becomes the growth-limiting factor throughout a very wide zone of experimentation within which the weight curve of the test animal becomes a function of the vitamin G intake. This basal diet need not be strictly free from vitamin G in order to serve the purpose of such determinations.

The basal diet which we now use is as follows (Diet 554); extracted casein, 18%; Osborne and Mendel[4] salt mixture, 4; cod-liver oil, 1 (or 2); butterfat, 9 (or 8); and 68% of starch on which has been dried the alcoholic extract of wheat in such proportion as to introduce the (80% alcohol) extract of 50 g. of whole wheat into each 100 g. of the air-dry food mixture.

The alcoholic extract used in this diet was prepared in the following manner: 800 g. of freshly ground whole wheat was shaken with 1500 cc. of alcohol, 80% by weight, for one and one-half hours, the extract separated by a Büchner filter and the residue treated with 1000 cc. of the 80% alcohol, filtered and washed with 300 cc. of the alcohol; the filtrates and washings were concentrated by evaporation (preferably under reduced pressure), finally dried upon starch and this incorporated into the food mixture as stated in the last paragraph. In adjusting the percentage strength of the alcohol, allowance was made for the moisture content of the air-dry ground wheat, so that the extraction should take place at an actual alcohol concentration of 80% by weight.

In the chief series of experiments employed in the development of this method, Diet 554 as above described was fed to rats from the age of four weeks. For about two weeks they showed slight gains in weight, probably due to bodily stores of vitamin G which they had possessed when placed

[3] F. C. Bing and L. B. Mendel, *J. Nutrition*, **2**, 49 (1929); H. C. Sherman and M. R. Sandels, *Proc. Soc. Exptl. Biol. Med.*, **26**, 536 (1929); and *J. Nutrition*, **3**, 395 (1931); D. L. Hussermann and R. A. Hetler, *ibid.*, **4**, 127 (1931); L. N. Ellis, Dissertation, Columbia University, 1931, not yet published.

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

upon the diet. When weight became constant this "fore-period" or "depletion" period was ended and the test period begun. A representative rat of each litter was continued on Diet 554 only, as a "negative control"; others received graded allowances of milk powder as source of vitamin G. The average results of thus feeding vitamin G at four different levels are shown in the accompanying graph (Fig. 1).

The gradual decrease in weight of the negative control animals may be taken as an indication that the basal diet is sufficiently free from vitamin G to be suitable for this work. At the lowest present level of feeding of vitamin G, designated on the chart by X, there was a slow gain; with successively higher levels of feeding the gain curves respond with very satisfactory regularity when sufficient numbers of cases are averaged. The curves shown are averages of eleven to sixteen cases each.

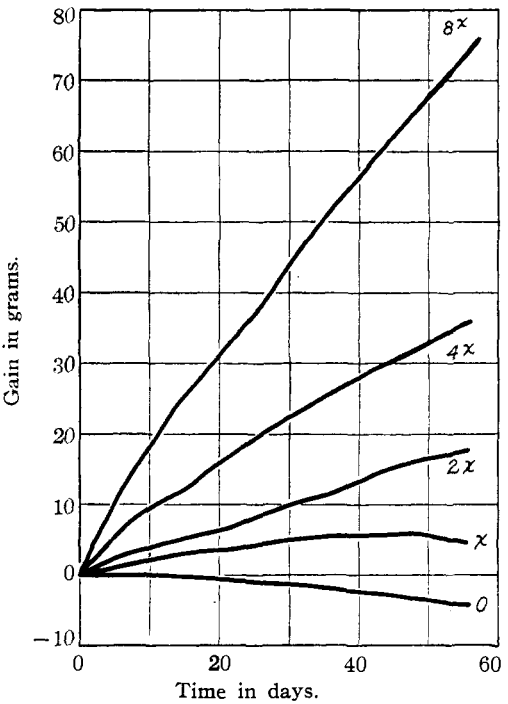

Fig. 1.—Average growth curves of rats receiving different amounts of vitamin G. The lowest curve shows the gradual loss of weight of the "negative control" animals which received the basal diet only; the others, the effects of feeding X amount per day of vitamin G in the form of milk and of 2, 4 and 8 times this amount, respectively.

Hence under conditions such as obtained in these experiments, this method affords a means of making quantitative measurements of vitamin G values of the same order of accuracy as in corresponding work with vitamins A and B;[5] and the same rate of gain used in these latter cases, namely, 3 g. per week during the test period, seems also satisfactory as a basis for expression of quantitative relations in dealing with vitamin G.

A "unit" of vitamin G might then be defined as that amount which when fed as a daily allowance induces a gain of 3 g. per week in an experimental animal standardized as here described and fed a basal ration which

[5] The prevention of coprophagy seems to be almost as important in work with vitamin G as with vitamin B. The use of cages with wide-meshed raised screen floors may well be supplemented by careful watching of the experimental animals.

is sufficiently freed from vitamin G to result in loss of weight during the test period.

In applying this method to the measurement of vitamin G (B_2) values of foods we must now safeguard against error by keeping in mind the fact that, using rats as experimental animals, the vitamin G (B_2) will continue to be the growth limiting factor only so long as the still more recently apprehended factor (or factors) needed for growth is (or are) amply supplied either through the food or by previous storage in the body of the test animal. In the experiments summarized in Fig. 1, the vitamin G was fed in the form of milk, which probably contains all the vitamins needed for mammalian nutrition. When the food or vitamin G preparation under test does not supply the "new factor," there may be expected a flattening of the gain curves (especially at the higher rates of gain) after the test animal has grown as much as his previous bodily store of the new factor permits.

In order to safeguard against possible error from this source when testing foods whose nature in this respect is unknown, it may be advisable to add to the method as here described a more explicit provision to ensure that the basal ration and the body of the test animal shall together supply enough of any "new" factor or factors to ensure that nothing else than vitamin G shall become the growth-limiting factor at any time in the test period employed for the measurement of vitamin G value. It is partly to safeguard against possible shortage of the thermolabile factor described by Reader[6] through its partial destruction during concentration of the vitamin B containing extract, that we now recommend the precaution of evaporating this extract at the low temperature permitted by the use of a highly efficient vacuum pump.

Until such time as all the factors required for the growth of young mammals are known, we cannot be logically certain that a given basal diet provides amply for all of them except the one under investigation; but this is only one of many cases in which research would be unduly retarded if we allowed our experimentation to be unduly inhibited by the remote possibilities of abstract logic. The internal evidence of the experiment will often on careful consideration suffice to safeguard the interpretation. In experiments such as those summarized in Fig. 1, the smoothness of the curves for the entire experimental period is a strong indication that the same limiting factor was operative throughout.

Granting that for the present our measurements of vitamin G values may depend upon the supplementation of what the basal diet furnishes by some unknown substance stored in the body of the experimental animal, there is a possibility that this latter substance might become the growth-limiting factor through the exhaustion of the bodily store; but there is

[6] V. Reader, *Biochem. J.*, **23**, 61, 689 (1929); **24**, 77 (1930).

also a strong probability that in such case the weight curve would show either a pronounced flattening or a point of inflection.

Since the working out of the method essentially as here described, it has been used in this Laboratory for the testing of various materials, including some laboratory preparations obtained in fractionation and concentration experiments such as might be expected to separate to a greater or less extent some of the unknown or newly-apprehended substances which accompany vitamin G in natural foods, and in several cases it has been found, as might be expected from the considerations above mentioned, that weight curves, especially at higher rates of gain, tend to flatten before the end of an eight-week experimental period. Here some substance provided by bodily store in the early part of the experimental period had probably become the limiting factor in its latter part. To prevent the possibility of the vitiation of the results from such a cause, the important precautions are to restrict the quantitative interpretation to such an experimental period as shows a fairly uniform rate of gain of weight, and to perform the experiments at such levels of vitamin intake as shall limit growth to about 3 g. per week and thus conserve the bodily store of the unknown substances or "new factors."

In general, the more regular the rate of gain, and the smaller the total gain, the less is the danger that the quantitative accuracy of the experiment will be injured by exhaustion of the bodily store of any growth-essential not yet sufficiently known to be provided in the basal diet. There should, however, be a sufficient length of experimental period to adequately establish the form of the weight curve, and a sufficient difference between the weight curve of the test animal and its negative control to permit of satisfactory quantitative interpretation.

As meeting this combination of criteria it seems well to base quantitative measurements and comparisons of vitamin G values upon experimental periods of from four to eight weeks during which there is a fairly uniform rate of gain of 3 g. per week.

The principles of the method here described may be followed without the employment of all of its details. For example, such laboratories as prefer an activated fuller's earth or a rice-polish preparation instead of the wheat extract which we have used as a source of vitamin B, may still make use of all the essential features of the method; and with satisfactory controls the results could be expressed in terms of the same units of vitamin G value.

Quantitative testing for vitamin G values cannot yet safely be formulated as a merely mechanical routine; but it can serve as a means of advancement of knowledge through carefully conducted and critically interpreted research.

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