The product polymerized rather quickly, and had to be used immediately to avoid loss.

Anal. Calcd. for C_8H_7Br: Br, 43.65. Found: Br, 43.29.

Dehydration of the carbinol with potassium acid sulfate gave a low yield of the styrene derivative.

m-Ethylbromobenzene.—A solution of 14 g. of freshly prepared *m*-bromostyrene in 50 cc. of alcohol was reduced in the usual fashion with hydrogen in the presence of platinum oxide–platinum black.[2] Twelve grams of *m*-ethylbromobenzene, boiling at 85–86° at 20 mm.; $n^{20}D$ 1.5470, was obtained.

An alternate method of preparation for *m*-ethylbromobenzene consisted of nitrating 100 g. of ethylbenzene by the method of Schultz and Flacksländer[4] to yield 70 g. of mixed ethylnitrobenzenes, boiling at 120–145° at 20 mm. The mixed nitro compounds (40 g.) were dissolved in alcohol (100 cc.) and reduced with hydrogen under three atmospheres pressure, using Rainey nickel[5] (5 g.) as a catalyst. The crude amine mixture was treated with acetic acid and acetic anhydride and brominated, the acetyl group was removed and the remaining mixed ethylbromoaminobenzenes were diazotized and treated with alcohol, as described for the corresponding preparation of *m*-bromotoluene from *p*-toluidine in "Organic Syntheses."[6] From 250 g. of ethylbenzene, 53 g. (12% of the theoretical amount) of *m*-ethylbromobenzene was obtained, b. p. 200–208°; $n^{20}D$ 1.5465; d^{20}_4 1.3493.

Anal. Calcd. for C_8H_9Br: Br, 43.21. Found: Br, 43.11.

(4) Schultz and Flacksländer, *J. prakt. Chem.*, [2] **66**, 153 (1902).

(5) Covert and Adkins, THIS JOURNAL, **54**, 4116 (1932).

(6) "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, 1932, p. 128.

Summary

p-n-Amyl-, *p-n*-octyl-, *p-n*-undecyl-, *p-n*-tridecyl- and *m*-ethylbromobenzenes have been synthesized, and these many intermediate compounds have been characterized.

URBANA, ILLINOIS RECEIVED APRIL 8, 1937

[CONTRIBUTION FROM THE BORDEN COMPANY, RESEARCH LABORATORIES]

Significance of Quantitative Relationships in Vitamin B Complex Studies[1]

BY R. C. BENDER AND G. C. SUPPLEE

The vitamin B complex is rapidly becoming resolved into a number of chemical entities which may be studied with precision and accuracy of interpretation from the biological point of view. The pure antineuritic factor (vitamin B_1) and pure lactoflavin (vitamin B_2 or G) employed as supplements in suitable basal rations have already served to expedite such research. Substitution of these pure supplements required for maintenance and for the promotion of growth, as well as purification of the gross ingredients of the basal diet, has established the existence of at least one other entity of the vitamin B complex, namely, the antidermatitis or antiacrodynia factor now commonly designated as vitamin B_6.

Vitamin B_1 and lactoflavin are known to be necessary for growth, and other things being equal growth rate of experimental animals is influenced, within limits, by the amount of these factors provided. It appears that vitamin B_6 may also influence rate of growth, but this cannot be stated as a firmly established fact, because it has not been isolated as yet. Notwithstanding this limitation, this factor may be demonstrated by the degree of protection which it provides against the acrodynia type of dermatitis and wherein the growth rate bears an inverse relationship to the incidence of dermatitis.

The data to be presented illustrate a simplified basic scheme for determining the influence of known amounts of pure vitamin B_1 and of pure lactoflavin upon growth rate and the influence of variable amounts of vitamin B_6 for the prevention of dermatitis and its concurrent effect upon growth rate. The significance of the interrelationship of each of these factors upon growth is also shown.

(1) Presented at the Symposium on the Vitamin B Complex, American Chemical Society, Chapel Hill, N. C., April 13, 1937.

Experimental

The basal ration employed was one previously reported from these Laboratories[2] and consisted of the following: vitamin free casein (Labco),[3] 20 parts; sucrose, 69 parts; hydrogenated vegetable oil,[4] 3 parts; salt mixture No. 40,[5] 4 parts; powdered agar-agar, 2 parts; and cod liver oil, 2 parts. White rats from twenty-two to twenty-five days old and weighing from 40 to 45 g. were placed upon this unsupplemented basal ration until initial growth had ceased and decline in weight or stationary weight had

(2) R. C. Bender, S. Ansbacher, G. E. Flanigan and G. C. Supplee, *J. Nutrition*, **11**, 391 (1936); S. Ansbacher, G. C. Supplee and R. C. Bender, *ibid.*, **11**, 401 (1936).

(3) Labco Vitamin Free Casein is distributed by The Casein Co. of America, Inc., Labco Products Dept., New York City.

(4) Crisco.

(5) H. Steenbock and E. M. Nelson, *J. Biol. Chem.*, **56**, 355 (1923).

been definitely established. A slight growth of a few grams for the first seven to ten days resulted and in all instances decline in weight or stationary weight was established at the end of the second week. At this time appropriate supplements were supplied depending upon the particular requirements of the experiment. The supplements employed in the various tests were crystalline vitamin B_1 (Merck), crystalline lactoflavin (Labco),[6] and desiccated rice polish concentrate (Labco).[6] All supplements were fed daily in aqueous solution of appropriate concentration. In those instances requiring autoclaved rice polish concentrate, the desiccated product was dissolved in water at a 7.5% concentration, the *p*H adjusted to 8.5 and the alkaline solution autoclaved for five hours at 120°.

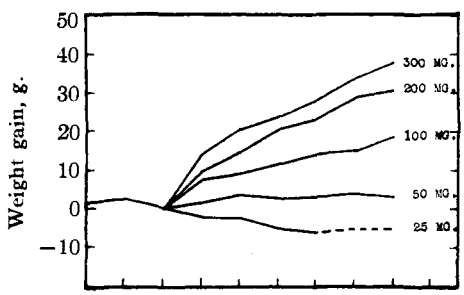

Chart 1.—Growth response from variable amounts of unautoclaved rice polish concentrate unsupplemented: added B_1, none; lactoflavin, none; r. p. conc. not autoclaved, variable.

The plan of study involved the determination of the effect of each substance fed in known amounts and also the effect of varying quantities of the combined supplements. The procedure resolved itself into three main divisions: the determination of the influence of variable amounts of pure vitamin B_1 in the presence of known amounts of lactoflavin and vitamin B_6 as carried by the lactoflavin- and vitamin B_1-free rice polish concentrate; the determination of the effect of different amounts of vitamin B_6 in conjunction with known amounts of pure vitamin B_1 and pure lactoflavin; and the influence of variable amounts of pure lactoflavin in conjunction with known amounts of pure vitamin B_1 and constant or variable amounts of vitamin B_6.

The records shown in the accompanying charts represent the composite results from 4 to 16 animals for each individual curve, with animals equally divided as to sex for each curve.

The data presented in Charts 1, 2 and 3 show the comparative results from the rice polish concentrate before and after autoclaving, with and without the addition of lactoflavin, and without added vitamin B_1. The 10 γ lactoflavin per day supplement caused an increased rate of growth when fed in conjunction with the rice polish concentrate at all levels. Chart 3 shows the results obtained from the autoclaved rice polish concentrate fed at the same levels as the unautoclaved product and supplemented with the same amount of pure lactoflavin. The autoclaving eliminated at least one factor essential for growth, presumably the vitamin B_1. The majority of animals died prior to the development of characteristic symptoms of polyneuritis.

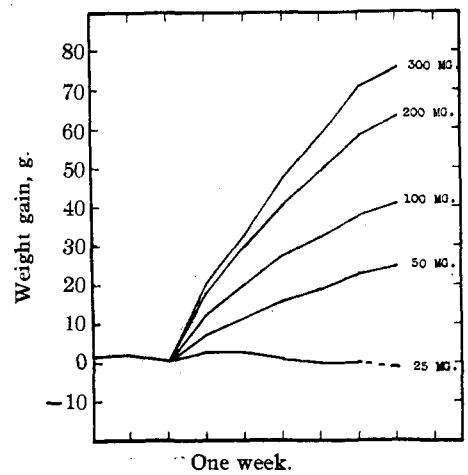

Chart 2.—Growth response from variable amounts of unautoclaved rice polish concentrate supplemented: added B_1, none; lactoflavin, 10 γ; r. p. conc. not autoclaved, variable.

It appears from the data in Charts 1 to 3 that the autoclaved rice polish concentrate and a constant amount of pure lactoflavin (10 γ per day) might be essential supplements for determining the influence of variable amounts of vitamin B_1 upon growth rate. In order to determine the validity of this concept these supplements were fed in conjunction with 2.5, 5.0 and 12.5 γ of pure vitamin B_1. The results from these comparisons show an increasing rate of growth with increased amounts of pure vitamin

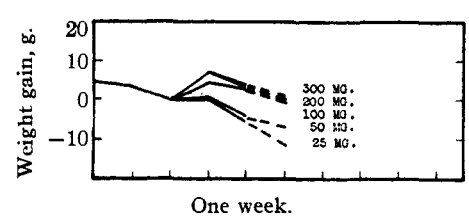

Chart 3.—Growth response from variable amounts of autoclaved rice polish concentrate supplemented: added B_1, none; lactoflavin, 10 γ; r. p. conc. autoclaved, variable.

B_1 throughout the 2.5 γ to the 12.5 γ range (Charts 4, 5 and 6). However, this increased rate of growth directly attributable to vitamin B_1 is less apparent from the lower levels of autoclaved rice polish concentrate, particularly at levels below 50 mg. per day. This relationship, interpreted in comparison with the data from the unautoclaved rice polish concentrate (Chart 2), suggests some factor in this material, other than vitamin B_1, which affects growth, even in the presence of the 10 γ lactoflavin and 12.5 γ vitamin B_1 supplements.

(6) Labco Lactoflavin and Labco Rice Polish Concentrate, desiccated, are distributed by The Casein Co. of America, Inc., Labco Products Dept., New York City.

Growth rate directly attributable to varying amounts of vitamin B_1 was determined in the presence of a constant amount of lactoflavin (10 γ) and a constant and seemingly adequate amount (100 mg.) of the autoclaved

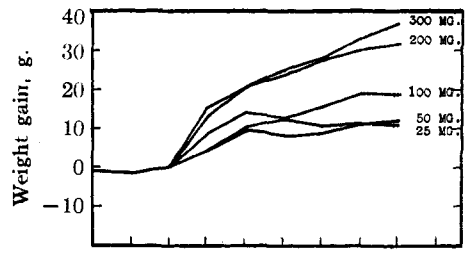

Chart 4.—Growth response from variable amounts of autoclaved rice polish concentrate supplemented: added B_1, 2.5 γ; lactoflavin, 10 γ; r. p. conc. autoclaved, variable.

rice polish concentrate (Chart 7). A remarkable consistency of relationship between growth rate and the amount of vitamin B_1 was shown. When no vitamin B_1

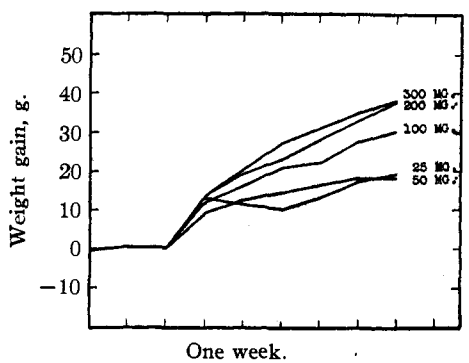

Chart 5.—Growth response from variable amounts of autoclaved rice polish concentrate supplemented: added B_1, 5.0 γ; lactoflavin, 10.0 γ; r. p. conc. autoclaved, variable.

was supplied, the animals immediately declined in weight and the majority died within a period of two weeks before manifestation of paralysis. At the 0.5 γ level the average

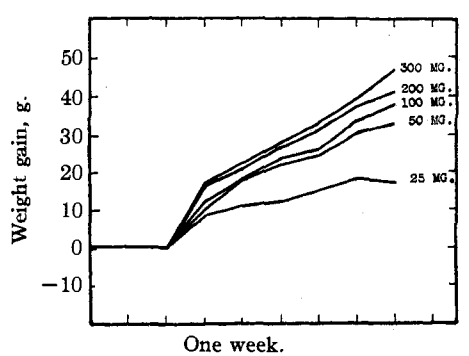

Chart 6.—Growth response from variable amounts of autoclaved rice polish concentrate supplemented: added B_1, 12.5 γ; lactoflavin, 10.0 γ; r. p. conc. autoclaved, variable.

length of life was prolonged, decline in weight was not so rapid and a substantial percentage of the surviving animals manifested polyneuritis symptoms. At the 1.0 γ level the survival period was further prolonged, weight remained substantially constant and some cases of polyneuritis were noted. At the 2.0 γ level the growth rate was slight but consistent throughout the six weeks observation period, averaging about 2.5 g. per week, no paralysis being noted. The higher levels of vitamin B_1 permitted an increased growth rate commensurate with the amount provided.

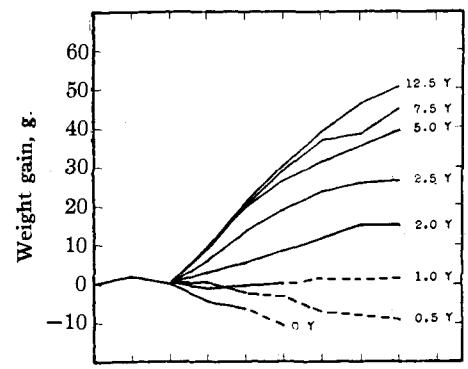

Chart 7.—Growth response from variable amounts of vitamin B_1 supplemented with added B_1, variable; lactoflavin, 10 γ; r. p. conc. autoclaved, 100 mg.

Since certain of the data already discussed have indicated a growth determining factor other than vitamin B_1 or lactoflavin in the autoclaved rice polish concentrate, attention was directed to a more intimate study of the

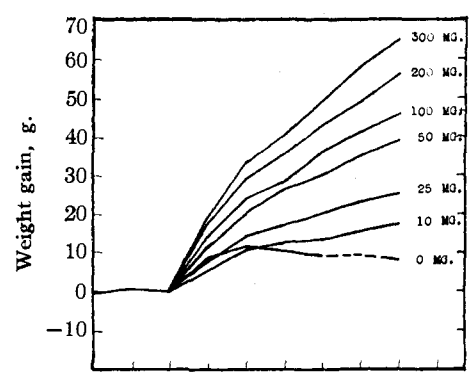

Chart 8.—Growth response and dermatitis from variable amounts of unautoclaved rice polish concentrate supplemented with: added B_1, 12.5 γ; lactoflavin, 10 γ; r. p. conc. unautoclaved, variable. Per cent. dermatitis: 300 mg., none; 200 mg., none; 100 mg., none; 50 mg., none; 25 mg., 25%; 10 mg., 63%; 0 mg., 100%.

effect of variable amounts of this material both unheated and autoclaved. A constant amount of vitamin B_1 and pure lactoflavin supplementing different amounts of the

unautoclaved rice polish concentrate varying from none to as high as 300 mg. per day, shows an apparent direct relationship between rate of growth and the amount of the unheated rice polish concentrate supplied (Chart 8). However, beginning at slightly below the 50-mg. level there was a consistent and progressive increase in the percentage incidence of the typical acrodynia type dermatitis, as the amount of the unheated rice polish concentrate supplement was decreased. No dermatitis was observed at the 50 mg. level and above. Similar supplementation with the autoclaved material gave a growth response at comparable levels only slightly lower than resulted from the unheated concentrate (Chart 9). The percentage incidence of dermatitis was only slightly greater at comparable levels; no dermatitis was observed at the 50-mg. level or above. These results are interpreted to mean that the factor affecting growth rate in autoclaved rice polish concentrate is vitamin B_6; when adequate amounts of this factor are provided by the concentrate growth is not significantly inhibited in the presence of adequate B_1 and lactoflavin provided as supplements in pure form.

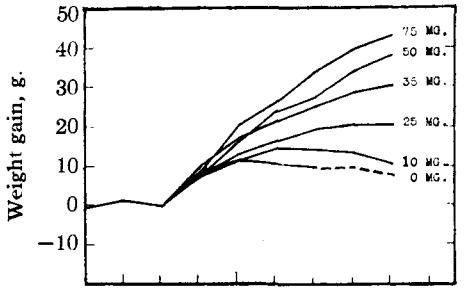

Chart 9.—Growth response and dermatitis from variable amounts of autoclaved rice polish concentrate supplemented with: added B_1, 12.5 γ; lactoflavin, 10 γ; r. p. conc. autoclaved, variable. Per cent. dermatitis: 75 mg., none; 50 mg., none; 35 mg., 38%; 25 mg., 69%; 10 mg., 88%; 0 mg., 100%.

These results direct attention to the apparent validity of the simplified basal ration supplemented with known amounts of pure vitamin B_1 and of pure lactoflavin, as a method for the biological assay of vitamin B_6, particularly wherein the results are interpreted on the basis of the percentage incidence of the acrodynia type of dermatitis. Since this condition is specific and clearly distinguishable, its presence may be used as the primary criterion, although the concurrent effect upon growth rate is also apparent.

The quantitative interrelationship of the various factors which affect growth as discussed thus far has been primarily concerned with the influence of variable amounts of vitamin B_1 and of vitamin B_6 accompanied by a constant amount of pure lactoflavin. The influence of variable amounts of pure lactoflavin upon growth rate has also been determined in conjunction with variable or constant amounts of the other two factors. One hundred milligrams of the autoclaved rice polish concentrate were used for supplying vitamin B_6. In addition to this essential supplement 2.5 γ and 12.5 γ of vitamin B_1 were also used as additional supplements. Variable amounts of lactoflavin ranging from none to as high as 20 γ per day were used. In the presence of 12.5 γ of vitamin B_1 there is a remarkably consistent relationship between the amount of pure lactoflavin fed and the rate of growth obtained throughout the range of levels used (Chart 11). However, when the same amounts of lactoflavin were fed with 2.5 γ of vitamin B_1, the growth rate is distinctly limited and the influence of variable amounts of lactoflavin is not as clearly distinguishable (Chart 10). It is obvious

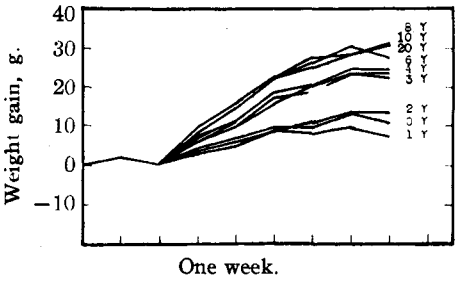

Chart 10.—Growth response from variable amounts of lactoflavin supplemented with 100 mg. of autoclaved rice polish concentrate and 2.5 γ of vitamin B_1.

that the low amount of vitamin B_1 is the factor which has limited growth rate. It has been reported[7] that rats deprived of lactoflavin become denuded and that this vitamin will prevent or cure the denuded condition. The animals in our experiments receiving no lactoflavin and those receiving up to the 6 γ level were retained on the test ration at the 12.5 γ level of vitamin B_1 for a period of 12 weeks. No evidence of the denuded condition as reported by other investigators was observed.

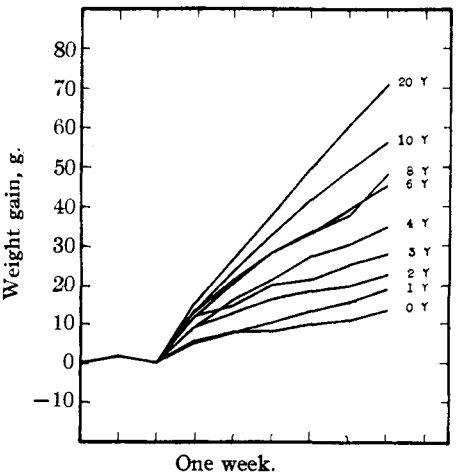

Chart 11.—Growth response from variable amounts of lactoflavin supplemented with 100 mg. of autoclaved rice polish concentrate and 12.5 γ of vitamin B_1.

The foregoing data tend to clarify the significance of growth rate and specific pathological

(7) L. R. Richardson and A. G. Hogan, *Mo. Agric. Exptl. Station. Research Bull.* 241 (1936); Paul György, *Biochem. J.*, **29**, 741 (1935).

conditions concerned in studies involving the vitamin B complex. Assay results for one of the known entities of the vitamin B complex, without knowledge of the relative amount of the other entities present under the particular conditions of assay, may be subject to wide variation and of questionable value, because of the influence of the amounts of the other entities present.

Simplification of the assay technique involving a simplified basal ration and the use of pure entities or proved substances as essential supplements will aid in the interpretation of results and extend their practical value.

While the subject of this paper has been presented primarily from the standpoint of the three known entities of the vitamin B complex, it is recognized that still other entities ultimately may be revealed. The data do not presume to present evidence in support of or as argument against the existence of factors other than those discussed. Any deductions of such character, based upon the evidence submitted, would be unwarranted, and in fact immaterial within the scope of these data. If other factors of the vitamin B complex exist and are of significance in influencing rate of growth or other conditions to which reference has been made in this paper, such a factor or factors are contained in the rice polish concentrate and have been operative in a comparable manner throughout the series of comparisons recorded. Invalidating and disconcerting contaminants are eliminated from this basal ration and obviously do not exist in the crystalline lactoflavin and crystalline vitamin B_1 supplements used.

Summary

A single simplified basal ration using sucrose as the carbohydrate may be employed, when properly supplemented, for the biological assay of vitamin B_1, lactoflavin (vitamin B_2 or G) and the vitamin B_6 or antidermatitis factor. Any one of these three factors may be detected, and its relative amount determined, by providing, as additional supplements to the basal ration, the other two factors, preferably at known quantitative levels.

The vitamin B_6 or antidermatitis factor may be assayed comparatively on the basis of the percentage incidence of the typical acrodynia type dermatitis by employing as additional supplements to the basal ration, pure and known amounts of lactoflavin and vitamin B_1.

Each of the three definitely established factors of the vitamin B complex affects growth rate to a readily distinguishable degree and, within limits, in proportion to the amount provided. However, the rate of growth as affected by the quantitative amount of any one of the three factors is significantly influenced by the amount of the other factors present.

BAINBRIDGE, N. Y. RECEIVED APRIL 12, 1937

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF WELLESLEY COLLEGE]

A Study of the Tautomeric Equilibria of Nitrosonaphthol–Naphthoquinoneoxime Systems[1]

BY H. S. FRENCH AND D. J. PERKINS

A limited amount of work has been done to determine the structure of aromatic nitrosohydroxy compounds by means of both physical and chemical methods. The earlier work[2] led to conflicting conclusions. Within the last eight years a renewed interest is indicated.[3] The improved methods of investigation have been more fruitful of results. It seemed especially worthwhile to extend the method of absorption spectra determinations, as it was used by Anderson[3b,c] and his co-workers on nitrosophenols, to the three isomeric nitrosonaphthols. Similar studies have been done recently on the analogous benzeneazonaphthol–naphthoquinone hydrazone system.[4]

Scheibe and his co-workers have shown that the absorption bands characteristic of negatively polarized groups are shifted in the opposite direction from those of positively polarized groups when the solvent is changed from a polar to a non-polar

(1) The material in this paper comprises a portion of a thesis presented by D. Jane Perkins to the Graduate Committee of Wellesley College in partial fulfilment of the requirements for the degree of Master of Arts, 1935.

(2) (a) Sluiter, *Rec. trav. chim.*, **25**, 8 (1906); *Ber.*, **44**, 1327 (1911); (b) Ilinski, *ibid.*, **17**, 2581 (1884); (c) Schmidt, *J. prakt. Chem.*, [2] **44**, 513 (1891); (d) Böniger, *Ber.*, **27**, 23, 3050 (1894).

(3) (a) Woroshtzow and Bogdanow, *ibid.*, **62B**, 68 (1929); (b) Anderson and Geiger, THIS JOURNAL, **54**, 3064 (1932); (c) Anderson and Yanke, *ibid.*, **56**, 732 (1934).

(4) (a) Burawoy and Markowitsch, *Ann.*, **503**, 180 (1933); *ibid.*, **504**, 71 (1933); Burawoy, *ibid.*, **509**, 60 (1934); (b) Kuhn and Bär, *ibid.*, **516**, 143 (1935).