VITAMIN B_1 IN THE NUTRITION OF EXCISED TOMATO ROOTS

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(WITH FIVE FIGURES)

Introduction

In previous studies of the growth-promoting materials obtainable from yeast and essential for the nutrition of excised tomato roots, these materials were shown to be soluble in 85 per cent. ethyl alcohol and insoluble in ether. Further extraction with absolute alcohol segregated two fractions both of which proved essential for optimal growth (**5**). The effectiveness of the insoluble residue left after absolute alcohol extraction of the 85 per cent. alcohol-soluble material was shown to be largely if not entirely attributable to the amino acids contained therein (**6**). The material soluble in absolute alcohol was not studied.

Besides the properties mentioned above, this material was shown to be stable to prolonged boiling in trichloroacetic acid, resistant to repeated drying, and resistant to moderately prolonged autoclaving such as was regularly employed in the preparation of nutrients (**4**). Further tests showed that its activity was completely destroyed by boiling for 2 hours in 1 N NaOH. Boiling for a similar period in 6 N HCl gave a product which supported apparently normal growth during the time necessary for a single passage (one week) but not for longer periods. The writer wishes to thank Dr. HUBERT S. LORING for carrying out these last treatments and for valuable advice during the conduct of the work.

The properties of this material as now known (solubility in 100 per cent. alcohol, destruction by alkali, and moderate stability in acid) are characteristic of vitamin B_1. It thus appeared possible that vitamin B_1 might be an important constituent of the fraction in question. Nevertheless, certain unpublished evidence obtained early in the conduct of this investigation seemed to oppose this view. Before any attempt was made to analyze the yeast material, a number of substances reported to act as growth stimulators was studied. These included inositol, vitamin B_1, heteroauxin, β-indolyl-propionic acid, haemin, yeast nucleic acid, glutathione, and a number of different sugars. None of these substances gave any evidence of stimulating growth of isolated tomato roots when tested alone. The effort to approach the problem in this way was therefore abandoned.

Nevertheless, out of the work already published there has grown the conviction that failure to demonstrate the effectiveness of any material when studied alone cannot be taken as conclusive evidence of its ineffectiveness in a complete nutrient. It seemed possible, therefore, that vitamin B_1,

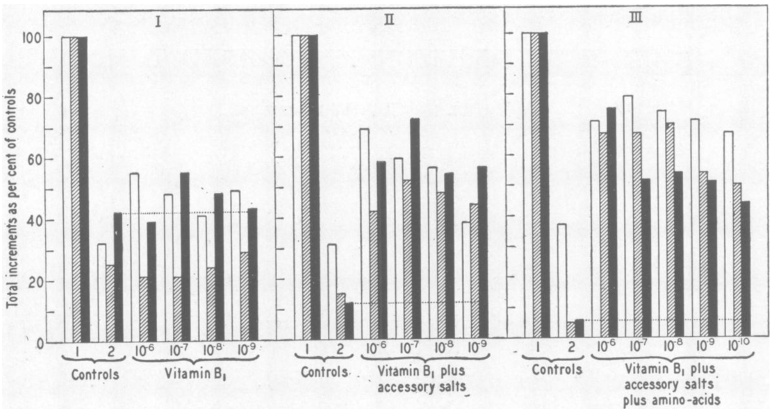

FIG. 1. Histograms showing growth of isolated tomato roots in nutrients containing standard salts, and sugar, with the addition in I of vitamin B_1 at concentrations of 10^{-6} [1] to 10^{-9}, of vitamin B_1 and accessory salts in II, and of these materials and amino acids in III. The open columns represent the first week's results (average of 20 cultures in each nutrient), the hachured columns the second week, and the blocked-in columns the third week. The transverse dotted line in each case is placed at the level of the negative control (without accessory organic material) in the third passage. All experimental increments must be above the level of this line to indicate growth stimulation. Control 1 is with yeast extract added to the basic nutrient, control 2 without accessory organic material of any sort.

although shown to be ineffective when taken alone, in the presence of the amino acid mixture developed in recently reported work (**6**) might prove to be of importance.

A study of the effects of vitamin B_1 in combination with these amino acids was, therefore, undertaken. While this work was under way the notes of BONNER (**1**) and of ROBBINS and BARTLEY (**2**) appeared, reporting evidence of the importance of vitamin B_1 in the nutrition of isolated roots. The present work is, therefore, a supplement to the work of these authors.

The basic nutrients, cultural methods, and materials used were the same as those previously described (**5, 6**). At the time these experiments were begun the roots were in the 196th passage.

Experimentation

Cultures were first carried out in the nutrient heretofore designated as basic, containing standard salts (including iron), accessory salts, and sugar (**5**), alone and with the following additions: amino acids only; vitamin B_1 only; amino acids and vitamin B_1 together; and yeast extract as control.

[1] This notation has been employed in the figures to conserve space. A concentration of 10^{-6} parts of vitamin B_1 in 1 part of nutrient is equivalent to 1 mg. per liter or 1 ppm. or 1 γ per cc.

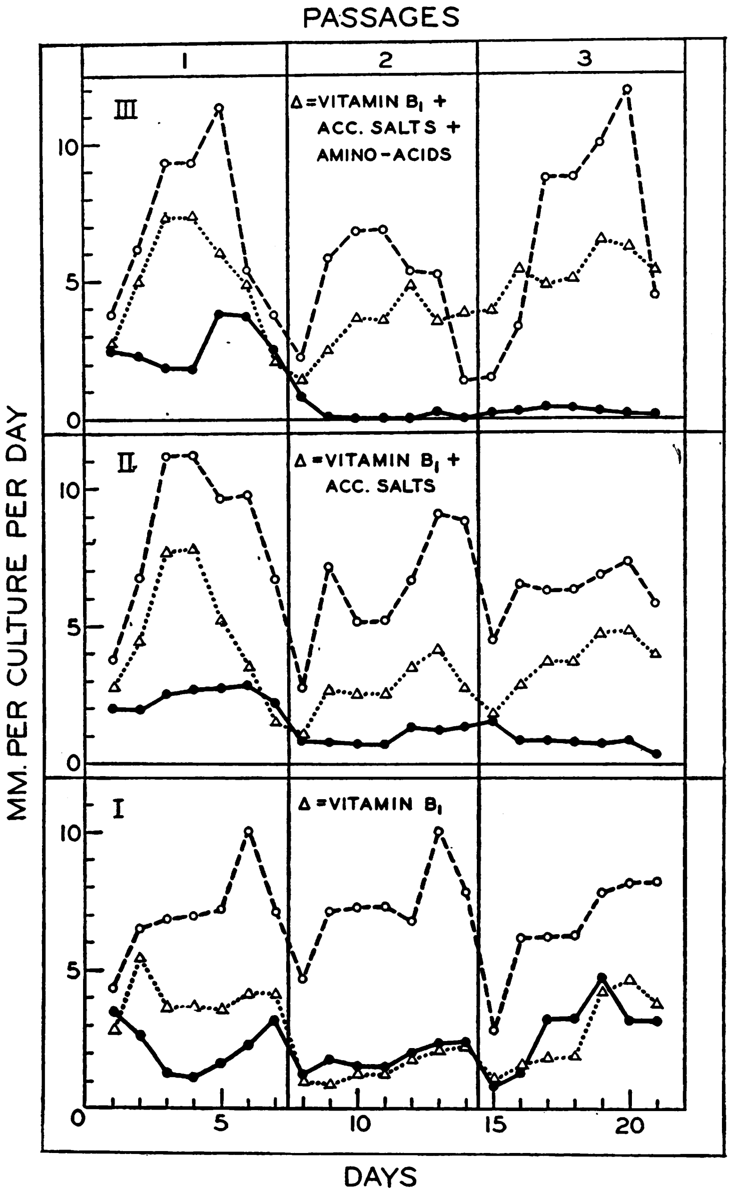

FIG. 2. Curves showing the growth rates of isolated tomato roots over three weeks' time in nutrients containing standard salts and sugar (solid circles) and with yeast extract added (open circles). The triangles represent growth in the same nutrient as that represented by the solid circles with the addition in I of vitamin B_1 at a concentration of 1.0 mg. per liter, of vitamin B_1 and accessory salts in II, and of vitamin B_1, accessory salts, and amino acids in III.

Vitamin B_1 was added at concentrations of 1.0, 0.1, 0.01, and 0.001 mg. per liter when tested alone, and at these and the additional concentration of 0.0001 mg. per liter when tested in combination with the amino acids.

When vitamin B_1 alone was added to the basic nutrient, a marked increase in growth rate occurred over the controls without accessory material. The results were, however, far from uniform or optimal (figs. 1–II, 2–II, and 4). In the first passage, growth at vitamin B_1 concentration of 1 mg.

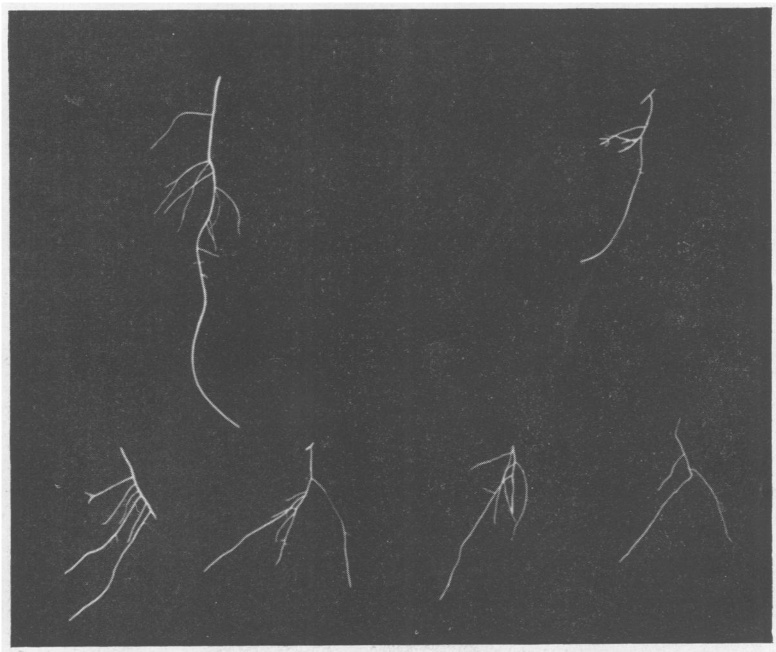

FIG. 3. Roots grown in a basic nutrient plus yeast (upper left), without any accessory material (upper right), and with addtiion of vitamin B_1 (lower row) at concentrations, reading from left to right, of 1.0, 0.1, 0.01, and 0.001 mg. per liter. Roots in the lower row are little if at all superior to the negative control. $\times$ 0.7.

per liter was equal to 69 per cent. of the control in yeast extract. The growth rates decreased regularly (59 per cent., 56 per cent., 38 per cent.) with decreasing concentration of vitamin B_1. In the second and third passages, a vitamin B_1 concentration of 0.1 mg. per liter gave the best results (52 per cent. and 72 per cent.). The roots were slender; most of the original growing points died so that growth occurred only in branches, and the branching was sparse. The condition of the cultures was obviously poor (fig. 4). A nutrient containing, in addition to the basic ingredients, only vitamin B_1, while capable of sustaining growth for a considerable period—

at least three passages—was clearly not satisfactory as a replacement for yeast extract.

This result agrees with that reported by ROBBINS and BARTLEY (**2**). The growth obtained was, however, much better than that observed two years ago, as mentioned above. In examining the records of the two sets of experiments for possible explanations of this discrepancy, two differences between the sets were noted. In the first place, the early tests were carried out with "crystalline vitamin B_1 Merck" of natural origin, while the later ones were conducted with "Betabion Merck," a synthetic product. In the second place, the later tests were made in a nutrient which included "accessory salts" (**5, 6**), while these were absent from the nutrient used (**4**) in the earlier tests. It seemed improbable that growth-promoting materials present in the synthetic vitamin B_1 might be absent from the natural product. The presence or absence of the accessory salts, therefore, seemed the more likely cause of the discrepancy.

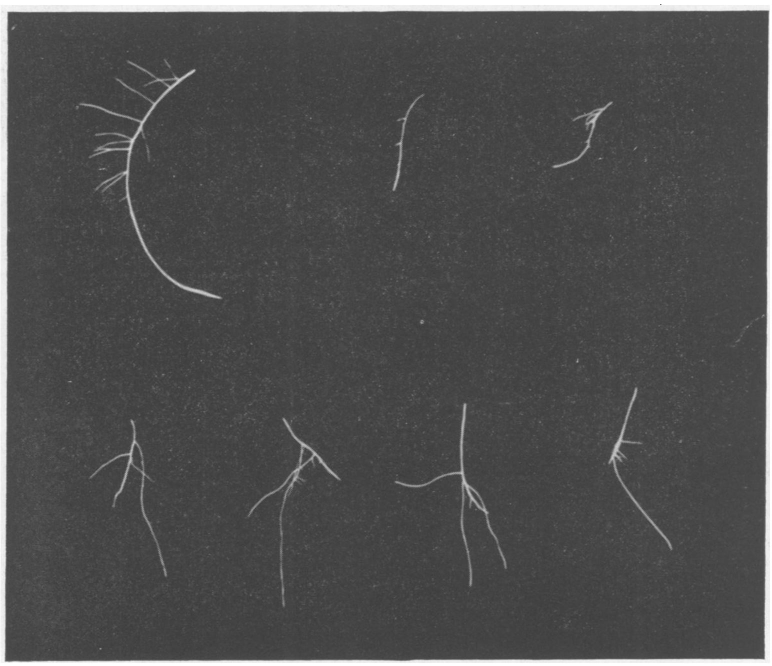

FIG. 4. Roots grown in nutrients similar to those used in cultures shown in figure 3, but with accessory salts added. Although the roots in the lower row are superior to the negative control (upper right), they are markedly inferior to those grown in the presence of yeast extract (upper left). It is clear that the tips in each experimental case had failed to grow and only a few branches were formed. $\times 0.7$.

To test this possibility, cultures were carried out in which vitamin B_1 was added at concentrations of 1.0, 0.1, 0.01, and 0.001 mg. per liter to a nutrient containing only the standard salts and sugar. In the first passage, while residual supplies of possible accessory salts carried over in the explant were still potentially available, the best growth, at a vitamin B_1 concentration of 1 mg. per liter, was equal only to 55 per cent. of the control (figs. 1–I, 2–I, and 3). In the second passage, growth at this concentration was only 21 per cent. of the control. In the third passage, growth at a concentration of 0.1 mg. per liter was equal to 55 per cent. of the control, but since the negative control, lacking all accessory material, likewise grew unusually well—42 per cent. of the control with yeast—this represents very little improvement over the completely deficient nutrient (figs. 2–I, and 3). From

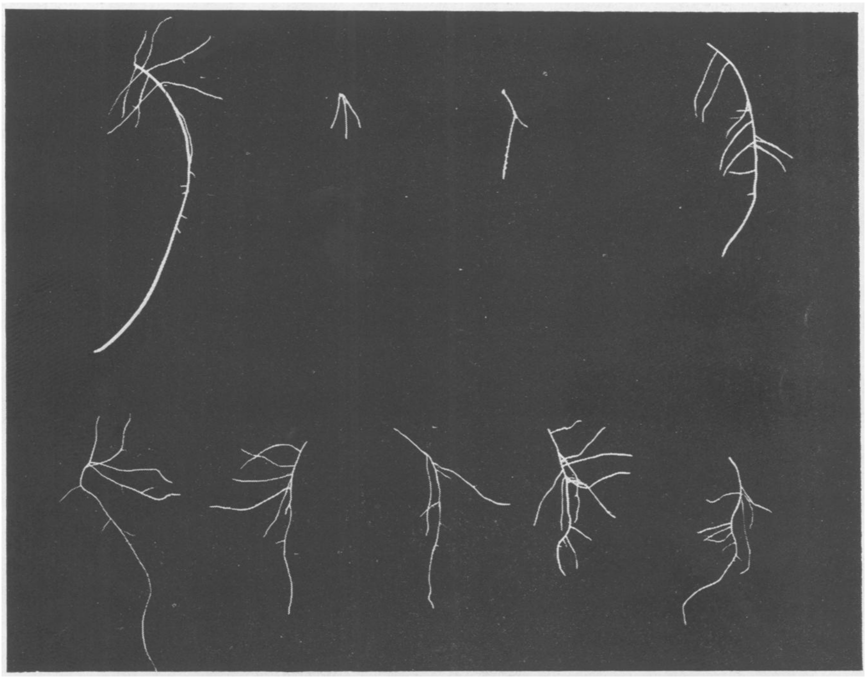

FIG. 5. Roots grown in the following nutrients: upper row, reading from left to right, control with standard salts, accessory salts, sugar, and yeast; the same but yeast omitted; the same but with an amino acid mixture substituted for the yeast; the same but with an absolute alcohol extract of yeast substituted for the usual yeast material; lower row, the same but with an amino acid mixture and vitamin B_1 at concentrations of 10^{-6} to 10^{-10} (from left to right) substituted for the yeast extract. The growing points of the experimental cultures have not died and the roots have branched quite profusely. While markedly superior to the roots grown without accessory organic material or with amino acids alone, or without accessory salts (fig. 3), they are still inferior to the controls provided with yeast. × 0.7.

these results it is clear that the accessory salts were the *limiting* factor, the vitamin B_1 being unable to induce growth in their absence. The omission of accessory salts, therefore, was, as postulated above, responsible for failure to detect the effect of vitamin B_1 in earlier experiments. These results are in agreement with those of ROBBINS, WHITE, MCCLARY, and BARTLEY (**3**).

When vitamin B_1 was similarly added to a nutrient containing the 9-amino-acid mixture developed in earlier work (**6**) in addition to the basic nutrient, the results were somewhat better than in a similar nutrient lacking the amino acids, but still far from optimal (figs. 1–III, 2–III, and 5). In all concentrations except 1 mg. per liter there was a regular diminution in growth rate from passage to passage, while at this concentration the growth rates in the three passages studied (71 per cent., 67 per cent., 75 per cent. of the control) were remarkably similar. The roots, while still slender and of reduced vitality (fig. 5), were much more profusely branched than when amino acids were omitted. Their color was better and the original growing points did not die. This nutrient, containing standard salts, accessory salts, sugar, vitamin B_1, and amino acids, appeared to be capable of sustaining continuous growth of tomato roots at a level somewhat inferior to that obtained with yeast extract.

Discussion

The work presented here has demonstrated that vitamin B_1 is of paramount importance in the nutrition of isolated tomato roots. This material is known to be present in yeast and is soluble in 100 per cent. alcohol. It was, no doubt, at least partly responsible for the activity of the yeast fraction extracted by this solvent. While concentrations of vitamin B_1 as low as 0.0001 mg. per liter gave a marked degree of stimulation, concentrations of 1.0 or 0.1 mg. per liter appeared to be somewhat superior. Since the yeast fraction soluble in 100 per cent. alcohol gave optimal results at a concentration of about 5 mg. per liter (**5**)—only slightly greater than this amount—it would appear that this fraction might consist largely of vitamin B_1. Yet vitamin B_1 was *not* capable of completely replacing the fraction. Addition of amino acids (**6**) improved the result but still did not bring it up to the control. The roots cultivated in such a nutrient, while growing at a fairly uniform rate, were slender, crooked, and of reduced vitality. Some other material, soluble in 100 per cent. alcohol and effective at concentrations of less than 1 mg. per liter, while not *essential* for growth, must be responsible for the residual activity of this fraction.

The nutrient developed in this work, while inferior to yeast extract, does appear to be *complete,* in the sense of supporting *continuous* growth of tomato roots. And its constitution, except for possible impurities in the

"C.P. grade" chemicals used, is known and can be duplicated. One of the primary tasks in the study of growth requirements of isolated roots—the establishment of a completely known control nutrient containing all necessary growth factors—has then been provisionally completed. This nutrient, containing standard salts, accessory salts, sugar, vitamin B_1 at a concentration of 1.0 to 0.1 mg. per liter, and a 9-amino-acid mixture at a total concentration of 10 mg. per liter, may be used as a standard in future work.

While this nutrient appears to be "complete," its obvious deficiency when compared to a yeast extract medium raises certain important questions which must be left for future work to answer. Is the deficiency qualitative or quantitative? If it is only quantitative, a careful examination of the present ingredients of the mixture at higher and lower concentrations than at present used should serve to determine the optimal quantities. It is probable that in this process one or more ingredient out of the 18 salts and 9 amino acids at present included may prove to be unnecessary. If, however, as is equally likely, the deficiency is qualitative as well as quantitative, it will be more difficult to correct. The synthetic nutrient may be supplying materials which are capable, partly but not completely, of replacing some yeast material. The substitution of selenium for sulphur or of pyrrol for iron would be similar cases. If such is the case, the optimal concentrations of these "Ersatz-" materials would still give results inferior to those obtained with yeast extract. No amount of quantitative adjustment of the nutrient will correct the deficiency or even clearly indicate which ingredient is deficient, and more complex methods will have to be devised for solving the problem. To determine which is actually the case, a quantitative study of all the present ingredients of the nutrient is, therefore, indicated as the next step in approaching these questions.

Summary

Experiments have shown that vitamin B_1, a probable constituent of the yeast fraction soluble in absolute alcohol, is an important and perhaps indispensable factor in the nutrition of excised tomato roots. The growth-promoting effect of vitamin B_1 is detectable only in the presence of the "accessory salts" which are also indispensable. While growth at a low level can be maintained apparently indefinitely in a nutrient containing only vitamin B_1, standard salts, accessory salts, and sugar, it is notably improved by the addition of a mixture of 9 amino acids.

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