Proceedings of the Iowa Academy of Science

Volume 47 | Annual Issue Article 17

1940

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VITAMINS IN PLANTS

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The particular part of this rather broad subject to which I want to call your attention this afternoon, concerns the synthesis of vitamins in plants, and some factors which influence their production. I shall illustrate from a number of experiments, including those which we have carried out at Iowa State College on the formation of vitamins A, B_1 and C.

Although some vitamins, for example A and D, may be manufactured in the animal body, the ultimate source of most of the vitamins is the plant kingdom. Animals get their vitamins either directly from plants, or indirectly by varying routes, through other forms of life. It should be recalled that green plants are not by any means the only source of vitamins, but that many of the lower forms such as yeast, molds, and bacteria also produce vitamins.

The importance of vitamins in animal nutrition has been recognized for a number of years, and the major emphasis has been placed on this aspect of their value. Recently, however, the significance of vitamins in the nutrition of the plant itself has been investigated, and it is becoming evident that the vitamins manufactured by the plant are essential for its own nutrition. It has also been suggested that the amount of vitamin produced by a plant might be modified by the conditions under which it is grown. It was this particular idea which interested me most and which I should like to discuss briefly this afternoon.

Provitamin A (carotin) seems to be a factor in plant growth. One theory relates it to photosynthesis and another to root formation, but whether there is any way of increasing the amount of carotin formed in the plant, or even whether the optimum requirements of a plant can be determined, is almost unknown.

Vitamin B_1 (thiamin) presents a similar case. It is produced by almost all bacteria, and yeast has been an important source of supply for years. Higher plants produce vitamin B_1 in varying quantities, but whether they would produce more if additional vitamins were supplied, is not known. From recent advertisements one would gather that it is only necessary to supply it to growing plants in order to realize all the glories of a seedsman's catalogue.

Last year it was found that the amount of B_1 in yeast could be altered by varying the conditions of growth. B_1 increased in the yeast crop if the medium was not aerated. It was also increased by additions of various extracts, including grain wort, liver, nucleic acid, and of vitamin B_1 itself.

Of vitamin C a little more is known. Its occurrence in seeds is rare, but it is formed in the seedling as soon as the seed sprouts. There is some evidence that the amount formed is affected by the nutrient supply — at least, maximum growth has been found to be associated with the maximum amount of C in the plant. The germination of wheat seeds and the initial growth of the shoots have also been increased by the application of vitamin C.

The problem of the variation in quantity of the different vitamins in plants and the effect of modification of growth conditions on the amount produced has considerable practical importance at the present time, even though some day it may have none — that is, if we feed the whole world on synthetic vitamins. For example, (and here the problem became one which concerned me as a soil chemist) does the organic matter of the soil have an influence on the quantity of vitamin formed in the plants? With the rapid decrease of available farmyard manure in recent years and the consequent resort to inorganic fertilizers, is it possible that we are decreasing the supply of vitamins in the crops we grow?

A few years ago the question was raised in southern India whether wheat and millet, the latter one of the principal food grains of that region, might vary in their content when grown on different soils. After some experimentation it was found that the B complex, and to some extent the vitamin A content, varied in the grain from different soils, and the variation seemed to be associated with the available organic matter in the soils. Grain from differently fertilized plots was tested, by feeding to rats. The B complex in the crop from plots receiving farmyard manure was distinctly higher than in the crop from plots receiving inorganic fertilizers. The grain from these differently fertilized plots given farmyard manure gave better crops than seed from crops grown with inorganic fertilizers.

From these tests the theory was developed that the microorganisms in the soil liberated from the organic matter some active constituent which stimulated the plant. The stimulant was believed to be either a vitamin or some pro-vitamin which caused the plant itself to produce vitamins.

The problem, however, may not be quite so simple. At the Ohio Agricultural Experimental Station the amount of vitamin B complex was investigated for wheat grown on experimental plots. Over a period of 35 years these plots had been treated each with a different type of manure or inorganic fertilizer. There was no variation in the B content of the grain that could be correlated with organic matter in the soil. There was some variation in the plots from year to year, and climate was suggested as a major factor for the differences rather than the soil.

At Iowa State College we (B. H. Thomas, E. E. Frahm and the author) have studied the production of vitamins in the plant *Lemna major* (Greater Duckweed). This plant lends itself readily to cultivation in nutrient media where freedom from contaminating microorganisms is essential. It is a small green flowering plant which normally produces by budding. The buds separate from the mother frond as they mature. Lemna can be grown very easily in nutrient solutions, entirely free from all organic matter, and has been grown in our laboratories for hundreds of generations, under controlled conditions of light and temperature, completely free from all organic material and also free from microorganisms.

The problems we set out to investigate were these: (1) Does organic matter affect the production of vitamins A, B_1 and C in Lemna? (2) Are bacteria or other microorganisms needed for the formation of these vitamins? In the Lemna produced on the inorganic material and under sterile conditions there could be no question of any carry-over of bacterial influence or of organic nutrient effects. The vitamin content of these plants was therefore compared with the vitamin content of Lemna grown with soil, that is, in the presence of organic matter and bacteria and other microorganisms.

The first test was for vitamin A. Plants were collected weekly, dried, ground and included in the basal ration of rats which had been fed an A-free diet and which were already showing xerophthalmia and cessation of growth. The rats were divided into four groups: (1) controls, on the A-free diet (2) rats receiving dry "sterile" Lemna (3) rats receiving dry "soil" Lemna, non-sterile, and (4) rats receiving fresh green "sterile" Lemna. The controls died, but all the rats receiving the Lemna in any form recovered quickly from the xerophthalmia and increased in weight. The growth curve for the rats on the dry sterile Lemna was identical with the curve for the rats on the dry "soil" Lemna, indicating no difference in vitamin A content, but those receiving the green plants put on weight faster than either. This difference was undoubtedly due to the loss of vitamin content in drying.

Two experiments about a year apart were made to determine the vitamin B_1 content of Lemna, rats again being used as test animals. As a considerable amount of time was needed to grow sufficient Lemna for rat feeding, the possibility of using cockroaches or the fruit fly, Drosophila melanogaster, was investigated. Some experimental work was done with the Drosophila, but without success. We therefore returned to the rat-feeding assays.

The first test for vitamin B_1 in the Lemna used 56 rats divided into groups, all of these having been fed on Sherman's B_1-free basal ration. Before the end of the experiment half the controls died. Toward the end, the remainder of the control group were given 5 International B_1 units a day and these recovered. All rats on the Lemna grown in the inorganic medium in the absence of microorganisms, and also those on the non-sterile "soil" Lemna, recovered and gained weight, and there was some indication that the sterile Lemna contained more of the B_1 per gram of dry weight than the "soil" Lemna.

The second experiment a year later with 29 rats, confirmed the presence of the B_1 in both sterile and non-sterile Lemna, and indicated that the organic matter and bacteria had not increased that vitamin in the plants.

For vitamin C, chemical instead of biological tests were made on the Lemna. Both iodine and 2.6 dichlorophenolindophenol were used. Extracts from the plants were obtained by acetic acid and trichloracetic acid. These extracts were all red in color and had to be decolorized by the mercuric acetate method before the reducing values by the iodine and the indophenol could be obtained. The presence of the vitamin C was indicated in both the sterile and the non-sterile plants, but quantitative results were somewhat irregular and did not make possible a comparison of the quantity in each.

In general the results of our tests tended to support the work at the Ohio Station. The experiments indicate definitely that it is possible for a green plant to manufacture the vitamins A, B_1 and C without the help of organic material and in the complete absence of microorganisms. It is quite possible that some forms of organic matter or extra supplies of the vitamins might have increased the amounts formed in the plant, but under our conditions the soil and bacteria did not have that effect.

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