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THE EFFECT OF VARIOUS GROWTH-PROMOTING SUBSTANCES ON THE REPRODUCTION OF LEMNA

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About 20 years ago, Bottomley (3), at the University of London, failed to secure normal growth of *Lemna* and other plants in inorganic salt solutions without the addition of organic matter. He concluded that special compounds, similar to vitamins for animals, were required by plants. These unknown substances were termed auximones. Mockeridge (20, 21) using solutions identical with those employed by Bottomley, found that a decided stimulation of the rate of reproduction of *Lemna* was produced by various nucleic acid derivatives isolated from soil and manure. Azotobacter and yeast were also found to contain accessories for plant growth, and the possible manufacture of auximones by bacteria was suggested. Opposed to Bottomley and Mockeridge were investigators who denied the essential nature of the auximones while admitting the stimulation factor.

At Iowa State College, Clark (4) successfully grew *Lemna* major in a strictly inorganic medium consisting of pure salts and water. These results with *Lemna* were confirmed by Saeger (22) and Wolfe (25) using different inorganic salt solutions, and by Ashby (1) with Clark's solution. Clark and Roller (8) later obtained continued reproduction of the *Lemna* in inorganic media in the absence of all microorganisms. The plants were healthy, and perfect in shape and color.

Nevertheless, certain types or extracts of organic matter seemed to stimulate plants — quickened growth, gave a fuller leaf or flower, or stimulated reproduction.

A number of organic substances (bioses) did the same for yeast. Extracts containing pantothenic acid were found to stimulate both the reproduction of yeast and the growth of alfalfa. Recently definite organic substances (auxins) formed in plants themselves were found to act as stimulants for plant growth. Certain vitamins (the essential dietary factors for animals) have also been found to promote plant growth and to be essential for continued growth of excised plant roots.

Bonner (2) suggested that all these various organic substances,

whatever the kind of plant (including bacteria or fungi) they stimulate, or whatever the type of stimulation, should be classed as plant vitamins; that plants be divided into either autotropic or heterotropic groups, depending upon their response. If an organic substance has to be supplied from an outside source, the plant is heterotropic, if no plant vitamin has to be supplied, it is autotropic. Many yeasts are undoubtedly heterotropic to some of the bioses. *Lemna* seems to be autotropic.

There is, however, the question of the degree or extent of the stimulation. If a plant will grow to maturity without organic matter, it would be classed as autotropic. If a certain organic substance increases its speed of growing or cuts down its time for maturity, the plant is certainly stimulated. In this case the plant could not be classed as completely autotropic or completely heterotropic. An example of this type is the variety of yeast which is stimulated by bios, but will grow and reproduce on a synthetic medium. (Fulmer, Nelson and White. 11).

Lemna major, a green plant, shows this duality. It usually propagates asexually by putting out a small frond or leaf which grows to the size of the parent plant and then separates. *Lemna* reproduces and grows to normal size in sterile inorganic media. Under these conditions a number of organic substances have a distinct effect on its growth and reproduction, including the pantothenic acid of Williams.

In 1933 Williams (24) obtained from various organic tissues an extract which stimulated the reproduction of yeast and was later found to increase the growth of alfalfa (McBurney, Bollen and Williams 17). Williams extracted beef liver and other organic matter with 80 per cent methyl alcohol and concentrated the extract by fractional electrolysis. His apparatus consisted of a series of cells with electrodes placed in the end cells; intermediate cells were connected by liquid junctions of the cell contents. With a high voltage a pH gradient was produced from alkaline reaction in the cathode, (pH 10-11) to acid in the anode, (pH 2.0-2.50). The pantothenic acid became concentrated in the acid cells, especially in the cell with a pH about 3.

This extraction-concentration procedure was used to prepare a concentrate of pantothenic acid for experiments with *Lemna*. Dry beef liver was extracted with 80 per cent methyl alcohol and the residue, following evaporation of the solvent, was taken up in distilled water. The water solution was electrolyzed in an eight cell apparatus with 1950-2000 volts, 1.0 to 0.1 milliamperes through

the cells. Varying amounts from the cell contents were added to Clark's solution (8) for *Lemna* at a pH 4.8.

The technique outlined by Clark (6) for growing sterile *Lemna* under controlled light and temperature was used throughout this and other investigations reported here. The effect of different nutrients on the plants is shown by changes in the slope of the growth curve (5) if the speed of reproduction alters; and in the form, color and size of the fronds or transformations in the roots if the vegetative material is affected.

Throughout the experiments the reproduction and appearance of *Lemna* grown in organic media (8) at a pH 4.8 was used for comparison, and this is referred to as the standard solution. For both standard and supplemented solutions the plants were transferred to fresh media every five days; at this time the fronds were counted and the number reduced to the initial quantity. Periodically during the growth period, nutrient agar slants were inoculated with plants from the sterile cultures to check for possible contamination; any non-sterile cultures found were discarded. As Williams (24) reported pantothenic acid to be heat stable in acid solution, the medium for *Lemna* was sterilized in the usual way after addition of the cell contents. No stimulation was shown by the inorganic residues after evaporation of portions of the cell contents and ignition of the dry matter.

Preliminary experiments showed the pH gradient established in the eight cells ran from 2.4 to 10.8. In the final experiments 1 cc. of the water solution (before electrolysis) of the extract contained the extracted material from 1.5 mg. dry liver. A medium containing 3 per cent of this unelectrolyzed solution increased the rate of reproduction of *Lemna*, but the plants were smaller and roots shorter compared with those in the standard inorganic solution.

After a 58 hour electrolysis of the extract of the liver, additions to the inorganic medium were made from each cell. From the growth curves of the *Lemna* there seemed to be no special concentration of the growth substance in any special cell, although in the medium containing 3 per cent of the most acid cell there was a more rapid reproduction than in the medium containing 3 per cent of the unelectrolyzed solution. But stimulation occurred on the cathode as well as on the anode side; a distinct increase in the rate of reproduction was obtained for plants in the medium containing 3 per cent of the extract from the most alkaline cell, and similar additions from the next two cells in line also gave a stimu-

lation. Although this increase in reproduction was not so great as that obtained for plants in cultures supplemented from the acidic fractions, it seemed that more than one plant vitamin was involved. Almost without exception, however, the increased rate of reproduction was accompanied by a decrease in size of fronds and roots, and in some cases in the amount of chlorophyll. Whether these effects were due to the same substances which caused the increase in reproduction, was not determined, but Kögl (12) and Thimann (23) found auxin *a*, a stimulator of oat coleoptiles, caused a decrease in root growth. McBurney (17) reported a lighter green color in alfalfa when stimulated by pantothenic acid.

The question arises whether the pantothenic acid can rightly be called a plant vitamin with regard to *Lemna*. Certainly it gives an increase in the rate of reproduction, but at the same time other functions are hurt. It is of course possible that under other conditions the reproduction might be increased and the other functions remain unaffected. At the present time it seems more in the nature of a stimulant than a vitamin if the latter is an essential to be supplied, without which the plant cannot attain perfect health and reproduction. It would be interesting to find if any function of yeast is inhibited due to increased rate of reproduction of that organism by pantothenic acid.

A situation similar to that of the pantothenic acid extract arose with auxin extracts. Roots and shoots of germinated corn were used as a source of these substances. Dollfus (10) and Kornmann (13) showed that corn seedlings were rich in auxins and Leonian (15) found corn roots contained similar substances which were soluble in 95 per cent ethyl alcohol.

Extracts were made with ethyl alcohol from both corn sprouts (up to one inch long) and roots (up to 2 inches long) and tried on *Lemna*. Attempts were made in each case to concentrate the auxins by fractional electrolysis in the same way as with the pantothenic acid. The growth methods for the plants were identical with those used for the experiments with pantothenic acid.

About 300 roots or shoots were used to prepare sufficient water solution for the electrolysis. Since this solution had a very low conductance, dilute phosphoric acid was added. This means was used by Williams (24) to increase conductance in the electrolysis of low-conducting extracts. At the end of 48 hours electrolysis a pH gradient was established similar to that recorded for the electrolysis of the extract of liver. Portions of the electrolyzed fractions

and of the unelectrolyzed solution equivalent to one shoot or root were added to media for *Lemna*.

For the shoots, stimulation of the rate of reproduction was quite marked from all the electrolyzed fractions of the extract, as well as from the unelectrolyzed solution. Again, there seemed to be no concentration of the auxin. For the root extract, the stimulation of the plant reproduction was not so great as from the cultures supplemented with material from the shoots, although the increase in the rate of reproduction left no doubt as to the presence of some material which speeded the increase of the *Lemna*. The substance appeared to be concentrated in certain cells of the electrolysis apparatus, especially in the last two anode cells, but there was no regularity.

In the media in which *Lemna* was stimulated by both shoot and root extracts, the increased rate of reproduction was accompanied by short roots, decrease in size and lighter color of fronds, as was the case with pantothenic acid.

A number of these auxins have been synthesized chemically. Indole-3-acetic acid (heteroauxin) was obtained from Dr. R. H. Manske of the National Research Council, Ottawa, Canada. Two other known growth-promoting compounds, phenylacetic and phenylpropionic acids, were obtained and used, in concentrations ranging from 0.0001 to 100 mg. per liter of medium with the sterile *Lemna* (Clark and Frahm 7). The cultures were prepared by adding water solutions of the compounds sterilized by filtration through Pasteur-Chamberlain filter candles to sterile salt solutions, and also by adding the substances to the inorganic media before autoclaving. At the concentrations used there was no destruction of the compounds in the slightly acid solutions, pH 4.8, by autoclaving at 20 pounds pressure for 15 minutes.

All compounds in concentrations of 0.1 mg. to 100 mg. per liter of media retarded the rate of reproduction of the *Lemna*. Lethal concentrations were found from 10 mg. up for indole-3-acetic and phenylacetic acids; with 10 mg. per liter of phenylpropionic the plants lived but were very small, light green in color and had very short, thick roots. The *Lemna* were similarly affected in concentrations of 0.1 and 1.0 mg. per liter of indole-3-acetic acid. For these concentrations of phenylacetic acid the plants were slightly larger and had a darker green color, while those treated with phenylpropionic acid were normal in appearance.

The changes produced in the *Lemna* by the non-toxic concentra-

tions of the compounds were not permanent; in all instances the plants, when transferred to standard inorganic solutions, appeared normal in about four weeks time. During this period there were no marked changes in the plants previously treated with indole-3-acetic acid, but the leaves of those which had been grown in the presence of 1 mg. per liter of phenylacetic acid and phenylpropionic acid increased markedly in size. However, the large fronds did not reproduce fronds of the same size.

The lower concentrations of the growth substances used, .01 to .0001 mg. per liter of media, neither increased nor decreased the rate of reproduction of *Lemna*. The appearance of the plants was normal except for lighter green color of the plants in the cultures containing .01 mg. of indole-3-acetic acid and for a slight shortening of the roots of the *Lemna* treated with .01 mg. per liter of the phenylacetic acid.

The action on *Lemna* of indole-3-acetic acid checks generally the results obtained from experiments with the same compounds by Leonian and Lilly (6) with detached roots and shoots of corn, of Lane (14) and Thimann (23) with oat seedlings and of Marmer (19) with wheat seedlings. Macht and Grumbein (18) obtained greater stimulation with *Lupinus albus* by intermittent application of the indole-3-acetic acid. This method was next used with *Lemna*.

Daily, except Sunday, *Lemna* were transferred, for a half hour period, from the standard sterile inorganic cultures to the solutions of the compounds which were renewed each week. Results with concentrations of indole-3-acetic acid from 2 to 10 mg. per liter were similar to those already recorded. No stimulation in rate of reproduction was shown, and fronds of the plants became small and roots decreased in length; but at concentrations of 0.1 mg. to 2 mg. per liter, a distinct stimulation of the rate of reproduction was obtained and the plants were normal in appearance.

For phenylacetic acid, a concentration of 10 mg. per liter gave no change in the plants; 5.0 to 0.1 mg. stimulated the rate of increase of the number of fronds, and 0.1 mg. gave a marked increase. Plants were normal in all instances except for a slight increase in root length of the *Lemna* treated with the 0.1 mg. concentration of the acid — which concentration gave the greatest increase in rate of reproduction. All concentrations used of phenylpropionic acid increased the rate of reproduction; here again the lowest concentration, 0.1 mg. per liter, gave the greatest stimulation. Plants were normal in appearance in each instance.

In this last series of the experiments, after five weeks of inter-

mittent treatment with the three compounds at a concentration of 1 mg. per liter, the daily exposure of the *Lemna* to the compounds was omitted; the rate of reproduction gradually decreased and in a few weeks had returned to a speed similar to that of the controls. The *Lemna* appeared normal and no changes took place during the gradual return to the rate of reproduction of plants in standard solutions. Therefore, under the conditions of intermittent treatment, the synthetic growth promoters proved capable of stimulating the increase in numbers of fronds of the plants, a stimulation without apparent injury to some other plant function. The more marked increase in the rate of reproduction was secured with phenylacetic and phenylpropionic acids rather than with indole-3-acetic acid, the synthetic heteroauxin.

It was earlier suggested in this paper that plants like *Lemna*, definitely autotropic to growth substances but stimulated by them, might be classed as partly heterotropic. The three auxins act as stimulants to growth and reproduction of the *Lemna*; they are not essential and they have a narrow range of concentration for effective action. This agrees with the conclusion of Macht and Grumbein (18) who compared the action of indole-3-acetic acid on plants to the action of drugs on human beings.

In such cases the term "plant vitamin" seems to be out of place if applied to the stimulating substances. If it is definitely shown that the substances (e.g., pantothenic acid) were manufactured by the plant and essential to it, then the term "auxin" or "plant hormone" seems preferable. Similarly, Clark, et al (9) have shown that sterile *Lemna* grown in sterile inorganic medium under electric light (the standard of comparison for the experiments previously reviewed) synthesizes vitamins A, B_1 and C. It is possible that these vitamins are as essential to the plants' life processes as they are to animals, but they do not have to be supplied to a plant, unless it is definitely heterotropic to them.

SUMMARY

1. *Lemna major*, grown in inorganic solutions under controlled light and temperature, and free from microorganisms, was treated with various growth substances.

2. An extract of beef liver (pantothenic acid) and extracts of roots and shoots of corn (auxins) increased the rate of reproduction of *Lemna*. The stimulation was accompanied by an inhibition of root growth and a decrease in both chlorophyll and size of fronds.

3. Indole-3-acetic, phenylacetic and phenylpropionic acids, when included in the inorganic medium renewed twice weekly failed to increase the rate of reproduction at concentrations of 0.01 mg. per

liter, or lower. Intermediate concentrations up to 10 mg. per liter were more or less toxic and retarded the production of fronds; ten milligrams per liter or slightly above, killed the plants.

4. Intermittent treatment with the compounds produced increased reproduction without any apparent harm to the plants. Indole-3-acetic acid was the least effective.

5. *Lemna*, while definitely autotropic, can thus be stimulated by auxins, and to that extent must be classed as partly heterotropic.

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