SPECIAL ARTICLES

"AUXIMONES" AND THE STIMULATION OF LEMNA BY ORGANIC MATTER

SOME time after the discovery that vitamins were necessary for the growth and well-being of animals, a claim was put forward by Bottomley and Mockeridge that similar substances were essential for the growth of green plants. These investigators found that Lemna (duckweed) failed to grow and reproduce continuously in two well-known salt solutions, but that if extracts of organic matter from peat, soil or manure were added, satisfactory results were obtained. From their experiments they concluded

> It is now established that plants, in their turn, require growth-promoting substances, or auximones; which in the case of the lower plants, are apparently manufactured by themselves, but which in the case of green plants, must be supplied from without. Since these necessary accessory substances are essentially organic in nature, their only possible source in the case of ordinary green plants is to be found in the organic matter of the soil in which they are growing.[1]

My attention was drawn to the work of Bottomley when I was studying the reproduction of yeast in synthetic media at Toronto. I found that it was possible to grow yeast without the addition of any organic matter except sugar, although reproduction was always better when particular organic extracts were added. Fulmer, however, proved conclusively that these "Bios"-containing extracts were stimulants and not essentials. It occurred to me that similar results might be obtained with green plants if they were grown in suitable inorganic solutions.

Some time later at Iowa State College I had the opportunity of going more fully into the auximone theory. It was necessary first to find an inorganic medium in which the Lemna would reproduce without the addition of organic matter. To this end Mr. Roller and I set out systematically to test salt solutions containing the essential elements; eventually we obtained an inorganic medium in which Lemna major reproduced and remained healthy without any added organic matter.[2] This showed that there are no *essential* organic substances needed for the green plant in the same way that vitamins are necessary for animals. I investigated shortly afterwards the pH required by the plants and the effects of duration and intensity of light.[3] In all cases the medium was made from carefully purified salts and special care was taken to have distilled water free from contamination. Recently Ashby, in London, has used this medium for the growth of Lemna minor and has confirmed our conclusions.[4]

These results, however, still left open the question of stimulation of green plants by organic matter. Mockeridge found stimulation with soil and manure extracts, even with an inorganic medium unsuited to the Lemna—the greater the degree of decomposition of the manure, the more stimulation was found. The theory was therefore put forward by Mockeridge that bacteria produce the substances which stimulate the plant: "The work of elaborating the growth-promoting substances is therefore thus relegated to the soil bacteria."[5]

We have found that extracts of manure, of soil and of alfalfa, as well as various "Bios" fractions which affect the growth of yeast, will increase the rate of reproduction of the Lemna when the pH is kept constant. There is a rapid rise in the rate, from less than one part per million of the organic matter in the solution, up to ten parts per million for the particular soil used, twenty for the manure and sixty for the alfalfa, although the alfalfa extract did not behave exactly like the soil and manure extracts. There is a maximum rate of growth over a considerable range, after which the addition of more extract makes the medium increasingly toxic, especially with the alfalfa. Ashby has found that stimulation occurred with 0.2 parts per million of organic matter extracted from horse dung, that it was not impaired by autoclaving and that the ash did not increase the growth rate.[6] He considers that the action of the organic matter is catalytic in nature, "since any addition above a minimum dose has no further effect upon the growth rate."

In these experiments great care was taken to keep the plants as free as possible from contamination with micro-organisms. Ashby, in his later experiments, sterilized his solution and changed it every forty-eight hours—in some of my experiments I changed every twelve hours.[2] It seemed to me, however, that there was always the possibility of a symbiotic relationship between the Lemna and bacteria in which the latter would make use of the organic matter. In order to do away with this possibility it was necessary not only to grow the plants in a sterile solution, but also to free them entirely from bacteria—a much more

[1] Mockeridge, *Biochem. Jour.*, 14: 432, 1920.
[2] Clark and Roller, *Soil Sci.*, 17: 193, 1924.
[3] Clark, *Jour. Phys. Chem.*, 29: 935, 1925; *Plant Physiol.*, 1: 273, 1926.
[4] Ashby *et al.*, *Ann. Bot.*, 42: 771, 1928.
[5] Mockeridge, *Ann. Bot.*, 38: 723, 1924.
[6] Ashby, *Ann. Bot.*, 43: 805, 1929.

difficult operation. It was to this problem that Mr. Roller turned his attention last winter.

Hansteen in 1898 had reported an attempt to sterilize Lemna by washing—testing for bacteria by the effect on egg albumen. We were quite unable to free the plants of bacteria by this method—whatever the effect on the albumen, some bacteria always grew on a nutrient agar. This would be expected, and we found in later experiments that plants which were bacteria free on the surface—no growth showing for five or six days on agar—when macerated, produced colonies abundantly.

In his sterilization experiments Mr. Roller tried ultra-violet light and a large number of antiseptics at various concentrations, and finally developed a technique which killed the micro-organisms both inside and outside the plant—no growth is developed on any medium yet tried for the bacteria whether the plant is macerated or not. We have grown the sterile Lemna through some twenty-five to fifty generations in the inorganic solution and they are reproducing normally and continue to look healthy. The first unexpected result was that the rate of reproduction of the sterile plants was greater than that of the non-sterile plants in the sterilized inorganic medium.

A second unexpected result was that the addition of sterile organic extracts of soil and manure (optimum concentrations) to the sterile inorganic medium with the sterile plants resulted in a decreased rate of reproduction compared with the sterile checks in the purely inorganic medium; the non-sterile plants, with the same sterile additions of organic matter, reproduced faster than the non-sterile plants in the sterilized inorganic solution—a confirmation of the work we have mentioned above, and as Ashby has also reported. Without bacteria the organic matter seems to depress the rate of reproduction, although we have not yet found whether this is due to a variation in the optimum concentration of the organic matter under the sterile conditions.

At the same time cultures were run in which the sterile plants had added to them, both in the sterilized inorganic solution and in the sterilized solution with organic extracts, an infusion of live bacteria from soil. The inorganic solution with the bacteria tended to decrease the rate of reproduction of the Lemna; where the organic matter was present the rate was greater than in the same solution without the bacteria, but it did not altogether overcome the original depression caused by the organic matter—the rate was distinctly lower than the checks for the sterile plants in the sterile inorganic medium.

In the sterile medium with sterile plants we have so far failed to get stimulation of the rate of reproduction of the Lemna by adding sterile organic extracts which certainly increase the rate when the plants are non-sterile. Details of this work with the effects on dry weight and ash will be reported later after some of the debatable points are examined further, but the present state of the problem is distinctly interesting.

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OCCURRENCE OF GERMANIUM AND ARSENIC IN METEORITES[1]

THE only record of a non-terrestrial occurrence of germanium is due to Rowland[2] who obtained spectroscopic evidence of this element in the reversing layer of the sun. Investigations carried on in this laboratory established the fact that germanium is of wide distribution in so far as the earth's crust is concerned.[3] On deciding to extend these investigations to meteorites, six specimens were selected representing siderites, siderolites and aerolites. The specimens were subjected to arc spectral excitation by a method described elsewhere,[4] and the spectrograms were examined in the range between λλ 3040 and 2530 angstroms. In all cases the germanium lines λλ 2651.6 and 2651.1 were definitely, though faintly, visible. These lines are extremely helpful in recognizing germanium: in addition to their being highly persistent they lie in a range where the dispersion of the prism spectrograph is considerable, thus minimizing difficulties due to overlapping of lines, especially when specimens of complex composition are examined.

On the basis of these observations it is to be concluded that traces of germanium are present in the following meteorites:

1. Siderite from Toluca, Mexico.
2. Siderite from Welland, Ontario. (Fell 6: 30 A. M., December 14, 1807.)
3. Siderolite from Admire, Lyon County, Kansas.
4. Siderolite from Llano del Inca, Atacama, Chile. (Found in 1888.)

[1] Presented at the forty-third meeting of the American Astronomical Society, Harvard College Observatory, January 1, 1930. The investigation upon which this announcement is based was supported by a grant to S. L. Boothroyd and Jacob Papish from the Heckscher Foundation for the Advancement of Research, established by August Heckscher at Cornell University.

[2] Rowland, *Amer. Jour. Sci.*, 41: 243, 1891.

[3] See Papish, *Econ. Geol.*, 23: 660, 1928; 24: 470, 1929. Additional work is ready for publication.

[4] See Papish, Brewer and Holt, *Jour. Amer. Chem. Soc.*, 49: 3028, 1927.