among the 100 untreated check plants, nor were any found among the 100 plants treated with lycorine.

Preliminary experiments on the effect of sanguinarine on mitosis in excised root tips of Lilium have indicated that its effect is similar to that of colchicine in producing shortened and split "C-chromosomes." A more detailed account of these studies will be published later.

THOMAS M. LITTLE
DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES,
BUREAU OF PLANT INDUSTRY,
BELTSVILLE, MD.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

BACTERIAL ACTIVITY IN DILUTE NUTRIENT SOLUTIONS[1]

MOST media designed for the growth of heterotrophic bacteria contain from 0.1 to 1.0 per cent. of organic matter, and it is generally claimed[2] that the minimum concentration required for their multiplication ranges between 0.001 and 0.01 per cent., or between 10 and 100 mgm/l. This is considerably higher than the concentration of nutrients generally found in nature in lakes, soil solutions or sea water. Sea water contains an average of 4 or 5 mgm/l of organic matter, much of which is fairly refractory to bacterial decomposition,[3] yet there is evidence that bacteria multiply and are otherwise active in sea water. In fact, the bacterial population may increase from a few hundred bacteria per ml of freshly collected sea water to several million after a few days' incubation in the laboratory.[4]

In order to approach the minimum quantity of organic matter which limits bacterial activity, an organic matter-free mineral solution was prepared. To it was added enough peptone to give concentrations ranging from 1.0 to 100 mgm/l, after which test-tube quantities were inoculated with a loopful of organic matter-free water containing from 10 to 100 living bacterial cells. After thoroughly mixing, loopful quantities were streaked on nutrient agar plates and the procedure was repeated at intervals of 24 hours. So few cells were introduced that rarely did any growth occur on the plates inoculated initially. However, after 24 hours' incubation at 22° C ten out of twelve of the cultures tested had multiplied enough to produce an abundant growth on the nutrient agar when loopful quantities were transferred. Since the controls were properly negative, the experiment showed that the cultures had multiplied in the most dilute of the peptone mineral solutions, although only the solutions containing more than 10 mgm/l of peptone were turbid. Similar results were obtained in dilute glucose ammoniacal mineral solutions.

It is not surprising that the dilute nutrient solutions do not become cloudy with bacterial growth because it requires around a billion cells per ml of the size of those being used to produce perceptible turbidity. Even if all the organic matter (1 to 10 mgm/l) were assimilated, there wouldn't be enough to give the requisite number of cells to produce a turbid solution. Moreover, many of the cells in dilute nutrient solutions grow attached to the walls of the test-tube.[5]

Quantitative results were obtained by inoculating glass-stoppered bottles filled with mineral solution treated with concentrations of glucose, ranging from 0.1 to 10 mgm/l. After different periods of time the bacterial populations were determined by plate count procedures and the dissolved oxygen content of the water was determined. The results showed that the bacteria multiplied in concentrations of glucose as low as 0.1 mgm/l and that this amount of glucose was completely assimilated in four or five days at 22° C. Ten to twenty days were required for the complete assimilation of concentrations of glucose as large as 1 to 5 mgm/l. Between 60 and 70 per cent. of the glucose was oxidized and the remainder was converted into bacterial protoplasm. Similar results were obtained with glycerol, ethanol, succinic acid and lactic acid. As will be elaborated elsewhere solid surfaces seem to facilitate the assimilation of dilute nutrients. Under proper conditions it is believed that concentrations of utilizable organic matter considerably smaller than 0.1 mgm/l will provide for bacterial multiplication.

CLAUDE E. ZOBELL
CARROLL W. GRANT[6]
SCRIPPS INSTITUTION OF OCEANOGRAPHY,
UNIVERSITY OF CALIFORNIA, LA JOLLA

PRESERVING PLANT VIRUSES IN VITRO BY MEANS OF A SIMPLIFIED LYOPHILE APPARATUS

MOST plant viruses are readily inactivated *in vitro*. This characteristic makes it difficult to interchange viruses with other workers for comparison studies. For this reason it has been considered desirable to devise some method of so treating a plant virus that its virulence could be retained. Since oxidative action

[1] Contribution from the Scripps Institution of Oceanography, New Series No. 173.

[2] Marjory Stephenson, "Bacterial Metabolism," Longmans, Green and Company, 1939.

[3] S. A. Waksman and C. L. Carey, *Jour. Bact.*, 29: 545, 1935.

[4] C. E. ZoBell and D. Q. Anderson, *Biol. Bull.*, 71: 324, 1936.

[5] C. E. ZoBell, *Jour. Bact.*, 33: 86, 1937.

[6] On sabbatical leave from Brooklyn College, Brooklyn, N. Y. Assisted by grant No. 555 from the American Philosophical Society.