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.

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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.

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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.

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

was considered to have an effect upon the longevity of viruses, experiments were designed to determine whether the absence of oxygen would prevent inactivation of certain viruses.

Two potato viruses, Y-virus and Canada streak virus, were used in these studies. These viruses when extracted in air have a longevity at 15° C. of about 72 hours and 120 hours, respectively.

In order to extract the plant juices in CO_2 a special metal box was constructed, in the front of which were two round holes to which rubber sleeves were attached, permitting the operator free movement with hands inside the box. Above these openings a pane of glass was inserted in such a manner that it could be easily removed, thus leaving an opening through which plant material and equipment could be placed inside the box. Solid carbon dioxide (dry ice) was placed in this container. After the CO_2 gas had replaced the air, the box was closed and it was ready for operation. Potato leaves infected with a virus were placed inside this chamber and crushed in a mortar. A few cc of extracted juice were then put into each of several glass tubes. After these tubes were covered with clamped rubber tubing, they were removed from the chamber and attached to a modified lyophile apparatus, which can be easily constructed.

This lyophile apparatus consists of a manifold made of an inverted 2-liter round-bottom flask (Fig. 1, A) from the sides of the bulb of which extend two

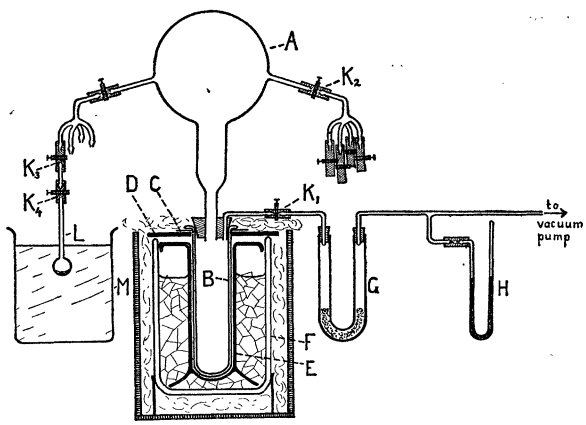

FIG. 1.

pieces of glass tubing. Each of these terminates in four outlets, to which tubes containing virus can be attached by means of rubber vacuum tubing. The sections of tubing K_3 and K_4, with screw clamps, allow removal of the tubes without releasing the vacuum. Four tubes L can be suspended in the air or be kept at a required temperature by submersion in a container M. The mouth of the flask A is fastened by means of a rubber stopper to a wide glass tube B used as a condensing chamber, which dips into a 2-gallon insulated vacuum jar F filled with a mixture of di-ethylene glycol and solid CO_2. The wire basket E permits easy removal of this chamber. The condensing chamber has a flared-out mouth which rests on a hard rubber ring D, which in its turn rests on the vacuum jar. The soft rubber washer C is used as a cushion. In the rubber stopper used to connect the manifold and condensing chamber, a glass tube (8 m.m.o.d.) is inserted which leads to a U-tube G filled with dryerite, to a manometer H, and finally to a vacuum pump. In order to facilitate cleaning, to prevent breakage and to aid in the detection of leaks, tubing and clamps K_1 and K_2 are used.

Dehydration of the virus was effected by a combination of evacuation, condensation and chemical drying, and resulted in formation of a thin film of solid particles inside the tubes L. The tubes were then clamped, removed from the lyophile apparatus, sealed and stored at room temperature. At monthly intervals a few tubes were broken and the contents of each used to inoculate 10 potato plants. Preparations of both the Y-virus and the Canada streak virus continued to produce 100 per cent. infection as long as 4 months after extraction and dehydration. Some of the tubes were improperly sealed and permitted air leakage; in all such cases the virus was invariably inactivated, indicating that oxidation had a direct or indirect effect on the destruction of the virus. Experiments are now in progress to secure additional information on this problem.

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