the mechanism necessary for the synthesis of biotin. It was necessary that pimelic acid have no effect on the growth of the organism used; otherwise, increased biotin production in cultures containing pimelic acid could be attributed to an increased growth of the cultures rather than to a conversion of pimelic acid into biotin.

The biotin free medium for yeast,[5] adjusted to pH 5.2, was used for culturing the mold. Sterilized 12 cc cultures (2 cc of addendum plus 10 cc of medium) were each inoculated with 2 drops of a suspension of *Aspergillus niger* spores. After 72 hours' incubation at 30° C., the cultures were autoclaved and filtered, and the biotin content of the filtrate determined by the yeast assay method.[5]

Results of a typical experiment, tabulated in Table I, demonstrate the activity of pimelic acid in promoting the synthesis of biotin.

TABLE I

Culture	Addendum per culture	Biotin content of filtrate of culture
1	None	0.006 microgram/culture
2	None	0.007
3	1 mg pimelic acid	0.096
4	1 mg pimelic acid	0.108
5	1 mg cysteine	0.011
6	1 mg cysteine	0.016
7	1 mg cystine	0.010
8	1 mg cystine	0.012
9	1 mg pimelic acid + 1 mg cysteine	0.192
10	1 mg pimelic acid + 1 mg cysteine	0.180
11	1 mg pimelic acid + 1 mg cystine	0.216
12	1 mg pimelic acid + 1 mg cystine	0.180

In spite of the difference in the amount of biotin produced, there were no visible differences in the growth of the cultures.

In subsequent studies, it was found that the maximum production of biotin could be obtained with pimelic acid concentrations of 20 micrograms per 12 cc culture.

The lower homologues of pimelic acid, succinic, glutaric and adipic acids, and an isomer, β-methyl adipic acid, were tested and found inactive. The higher homologues, suberic and azelaic acids, however, have activity comparable to pimelic acid. The biotin active substance produced from any of the active dibasic acids react in the usual manner with avidin.[6]

Cysteine or cystine, sources of organic sulfur, were found to enhance the effect of pimelic acid. A study of the supplementary action of these and other sulfur-containing compounds has given erratic results, but this problem is being investigated further.

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[5] E. E. Snell, R. E. Eakin and R. J. Williams, *Jour. Am. Chem. Soc.*, 62: 175, 1940.

[6] The physiological relationship between pimelic acid and biotin has been demonstrated independently by du Vigneaud, Ditmer, Hague and Long, who have shown that biotin is a growth stimulant for the diphtheria bacillus in the absence of pimelic acid.

TETRAPLOIDY IN ANTIRRHINUM MAJUS INDUCED BY SANGUINARINE HYDROCHLORIDE

IN the fall of 1941, Dr. Glenn A. Greathouse, discussing his work on the influence of alkaloids on the growth of fungi, mentioned that the alkaloid sanguinarine had produced peculiar swellings in the hyphae of certain fungi. This observation led the author to try the effect of sanguinarine on seedlings of *Antirrhinum majus* to determine whether it would produce polyploidy in a manner similar to colchicine.

In December, 1941, 100 seedlings of Antirrhinum (snapdragon, variety White Prosperity) were treated by placing a drop of 0.2 per cent. sanguinarine hydrochloride solution on the terminal growing point of each. At the same time, 100 seedlings of the same variety were treated with 0.2 per cent. colchicine, another 100 seedlings were treated with 0.2 per cent. lycorine (tried because, like colchicine, it is derived from monocotyledonous plants), and another 100 seedlings were left untreated for a check.

The toxic effect of the sanguinarine was very obvious within 24 hours after treatment, practically all the seedlings showing some dead tissue where the drop had been applied. At first the growth of the seedlings was greatly retarded, but after several weeks normal growth was resumed and the plants were examined for abnormalities of the leaves or stems. Eighteen of the plants were selected as appearing somewhat abnormal, and these were repotted for growing to maturity. While these plants appeared to have larger and thicker leaves than normal, the leaves had none of the roughened or wrinkled appearance characteristic of the seedlings treated with colchicine. Of these 18 plants, 9 were lost, due to an error in handling, but the remaining 9 were grown to maturity, and chromosome counts were made from propionocarmine smears of the pollen-mother-cells. Five of the plants were found to be tetraploids, and the remaining four diploids. Two of the tetraploids had some diploid branches, which had emerged below the point of treatment. Because some of the plants were lost, we can only say with certainty that tetraploidy was induced in at least 5 per cent. of the treated plants. This compared favorably with the results from 100 seedlings treated with colchicine, in which 4 tetraploids were found (a much higher percentage of tetraploidy has been induced by colchicine, however, using the same method of treatment, but repeating it 3 or 4 times at 3-day intervals). No tetraploids were found

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.

[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.