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Growth of Neurospora.

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Abstract

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The exercise described below is a portion of a laboratory period dealing with the growth of cells and cell populations in an introductory biology course for undergraduate students. It has given good results in the two or three years that it has been used at Dartmouth College.

Growth of Neurospora (students work independently).

Select from the instructor's table a Petri plate containing a nutrient agar. The surface of this agar was inoculated a few hours earlier at the point marked "X" with a small amount of mycelium of a wild type strain of the fungus Neurospora. After an adjustment period the hyphae began growing outward, giving a series of radiating mycelial strands. Hold the plate up to the light and note the area of growth with the aid of a hand lens. Select a large straight filament at the edge of the growing region. Using a grease pencil or other marking device, mark the bottom of the dish under this filament, so that you will be able to find it again. Now place the dish, still covered, upside down on the stage of your microscope, so that you can examine this growing filament by looking through the dish bottom and the layer of agar.

The method used to measure the growth rate of these cells is as follows: Inside the ocular of your microscope has been mounted a piece of film bearing 100 regularly-spaced marks. This "scale" is in the focal plane of the ocular, and thus will be superimposed on any object seen through the microscope. To measure the growth rate of the Neurospora filament, the filament must be brought into focus (low power) and the ocular (holding the scale) must be rotated so that the growing tip of the filament moves along the scale. The growth rate of the filament can then be determined in scale divisions per minute. A stage micrometer can be used to determine the size of the scale divisions in microns. Then the growth rate can be expressed in microns per minute.

After you have aligned a growing filament with the scale as described above, begin recording in the table provided in the manual the exact time, in minutes and seconds, at which the growing tip crosses each successive scale division. Continue these measurements for 10 minutes, or until the tip has grown across 10 scale divisions. (Smallest ocular micrometer division about 15 microns.) Plot these measurements on graph paper, using time on the horizontal axis and position (scale divisions) on the vertical axis. Draw a smooth curve through the points.

Questions: Is the growth rate constant? If so, calculate the average growth rate in divisions per minute and then convert to microns per minute. Enter calculations in the table.

Having measured the normal growth rate of the Neurospora filament, you are now asked to study the effect of one of a number of externally applied substances on the growth rate of this same filament. A list of the available agents will be posted on the board. (Among those substances which have been tried are sucrose and other carbon sources, sorbose, glycerol in various concentrations, urea, toxicants like dithiocarbamates, mercurials of various types, some amino acids, yeast extract, dinitrophenol, copper salts and many others.)

Make sure the filament you have been studying is marked so that it can be found again and then remove the plate from the microscope. Remove the cover and place one drop of the substance to

be tested on the marked filament. Replace the cover and center the filament under the microscope as before. As before, record the time the filament crosses each division of the ocular scale. Enter these data in the table. Continue these readings for 10 minutes or until the tip grows across 10 divisions. Plot the growth of the treated filament in the same manner as you did that of the untreated one. Connect the points with a smooth curve.

Questions: Is the growth rate of the treated filament constant? If so, calculate the average growth rate in divisions per minute and microns per minute and enter calculations in the table. If the growth rate of the treated filament is not constant, describe the nature of the growth curve. How would you describe the effect of the substance you used on the growth of a Neurospora hypha? How could you prove that the effects observed were due to the applied substance and not due to the water in which it was dissolved? How could you show that the effects observed were not caused by the brief removal of the cover? If you have time, perform experiments designed to answer these questions.

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