IN THE LABORATORY

Methods for Increasing the Value of Hydra as Material in Teaching and Research

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In the course of an investigation in which *Hydra fusca* was used it became necessary to secure known and controllable environmental conditions in which the organism would exhibit normal behavior. This necessitated discarding the usual "pond water" and substituting a known solution as the environmental fluid. During the progress of trials to this end certain observations were made which may prove of use in facilitating classroom culture of, and demonstrations with, this form and also in enhancing its value as experimental material.

Hydra from cultures collected locally or from cultures obtained from Woods Hole, when transferred to a saline solution[1] made with ordinary distilled water from the laboratory supply, went into "depression" (*i.e.* exhibited continuous contraction followed by loss of tentacles, leading often to final disintegration), beginning a few minutes after transfer.

If distilled water of great purity, triple distilled from glass, was used, this did not occur, and the hydra remained normal. Neither did it occur immediately if a small amount of the culture fluid was added to the saline made with the ordinary distilled water. However, the addition of crystalline egg albumin, at the rate of 0.2 cc. of a 3 per cent albumin solution/100 cc. of the saline, was found to give complete protection for several days (3 at least), under survival conditions.

Experiments indicate that the toxic action of the water is probably due to substances such as traces of heavy metals, which are effective in extremely low concentration. Copper was found to be toxic within a few hours when present in a molar concentration of 5×10^{-7}. The action was completely prevented by albumin at 1:50,000, possibly even less. Tests indicate that this protective action is most probably the result of removal of the toxic material by adsorption on, or combination with, the albumin.

Hydra in solutions containing sufficient albumin show very fully stretched bodies with the tentacles extended to their maximum length, giving a display seen only rarely.

While the foregoing treatment will eliminate the need for exceedingly pure water in making cultures or class demonstrations, it was realized that a preparation of crystalline egg albumin is not readily available to all, so a few experiments were tried using filtered egg white. This will serve for classroom use. The amount needed will have to be ascertained by trial and error.

It is not necessary, at least over periods of a few hours, to use a saline solution. Distilled water shaken with a little egg white will serve to maintain the exhibition.

Another demonstration readily made under these conditions is vital staining with methylene blue. It was found that by increasing the concentration of albumin the toxic action of the dye was so reduced that deeply stained specimens showing little, if any, symptoms of damage could be made. For this the albumin concentration should be increased about 5-fold. A solution of the dye of 1:30,000 is tolerated. If, after staining, the hydras are kept for 12 hours in a solution lacking the dye, all the dye will be found in the endoderm, leaving the ectoderm colorless except for the cnidoblasts. These show as minute, dark blue dots.

Hence, by the use of these simple techniques, classroom cultures may be kept more easily, and demonstrations of the phenomenon of depression, recovery therefrom, the beauty of the fully extended animal, and the tissue layers may be easily made in the living state.

The ability to keep hydra in good condition in a known environmental medium will greatly increase its value as research material.

[1] The saline contained KCl, 0.002 gram; NaCl, 0.05 gram; $CaCl_2$, 0.003 gram; and $NaHCO_3$, 0.002 gram/1,000 cc. distilled H_2O.

Electron Micrographs of X-Ray–treated *Escherichia coli* Cells

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Ever since Muller (*14*) produced mutations in *Drosophila* by X-rays, this method has been adopted in the study of mutations in many other organisms, including microorganisms. Beadle's work (*1*) on X-ray mutants of *Neurospora* has not only given us many new ideas of the metabolism of this fungus, but it has also been a contribution to fundamental ideas of genetics. Euler, Ahlstrom, and Hogberg (*5*) X-rayed yeast cells to produce genetic changes, including formation of giant cells.

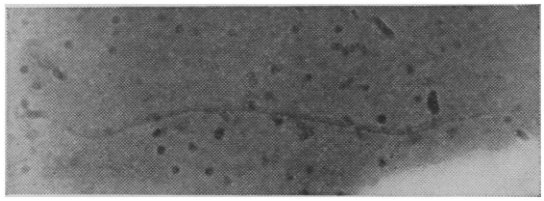

FIG. 1. X-ray-treated *E. coli* cell which has become elongated (ordinary microscope).

The effect of X-rays on bacteria has been studied by a number of investigators since shortly after Roentgen's discovery of the rays. Newcomer (*15*) in 1917 noticed that X-rays had a bactericidal effect on *Eberthella typhosa*. Clark and

Boruff (*2*) measured the lethal effect on *Bacillus coli* and *Erythrobacillus prodigiosus*, as well as destruction of the ability of the latter to produce the characteristic red pigment. Drea (*4*) attenuated *Mycobacterium tuberculosis* by the use of X-rays. Several geneticists, including Demerec (*3*), have used X-rayed bacterial cells in their studies on the phenomenon of mutation.

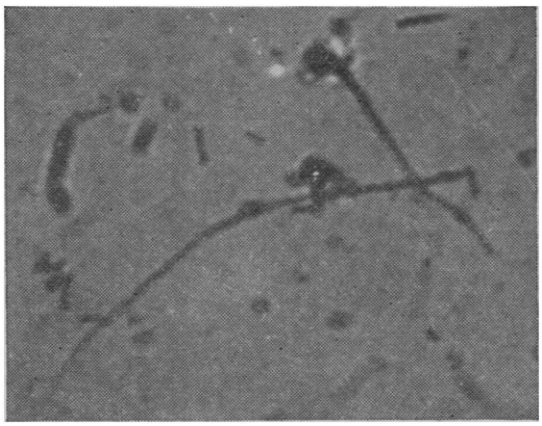

FIG. 2. X-ray-treated *E. coli* cells which have become elongated (ordinary microscope).

Lincoln and Gowen (*13*) studied several mutations produced by X-rays in *Phytomonas stewartii*. Haberman and Ellsworth (*8*), in a similar investigation on *Staphylococcus aureus* and *Serratia marcescens*, noticed the formation of elongated cocci into chains, instead of the usual grape-like or packet groupings.

It has been observed, in X-ray–treated bacteria, that the organisms sometimes lose their ability to multiply but are not dead. As early as 1917, Kempster (*10*) noticed that his X-rayed

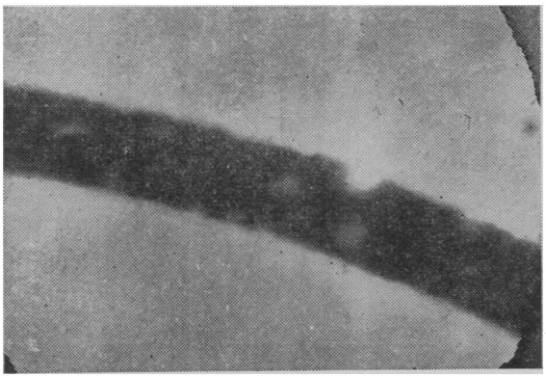

FIG. 3. Section of X-ray-treated *E. coli* cell which has become elongated (electron micrograph). Note mottled appearance.

bacilli were not destroyed, although they failed to multiply. Often, these X-rayed bacteria which have lost their ability to multiply continue to grow into unusually elongated cells. Lea, Haines, and Coulson (*12*) made a careful study of these giant bacteria, and Evelyn Wilkin (*17*) observed the phenomenon in her mutation studies of *E. coli*. Explanations for cell elongation after irradiation, without division, are offered by Hollaender (*9*), Rahn (*16*), and Lea, Haines, and Bretcher (*11*).

Gray, Marton, and Tatiun (*7*), who made an electron microscope study of X-ray–produced mutants of *Acetobacter*, observed bulges in the mutants, which they interpreted as high concentrations of nucleoprotein.

The purpose of our investigation was to examine these irradiated, elongated cells under the electron microscope and study the ways in which they might differ from normal bac-

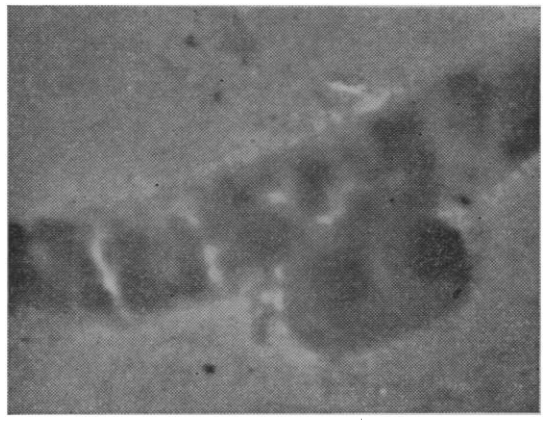

FIG. 4. Section of X-ray-treated *E. coli* cell (electron microscope). Note irregular appearance.

terial cells. Irradiation at 50 kv. and 20 ma. produced the irregular morphology. Figs. 1 and 2 show elongated *E. coli* cells as they appear under the ordinary microscope. Except for the elongation, the appearance is the same as that of the

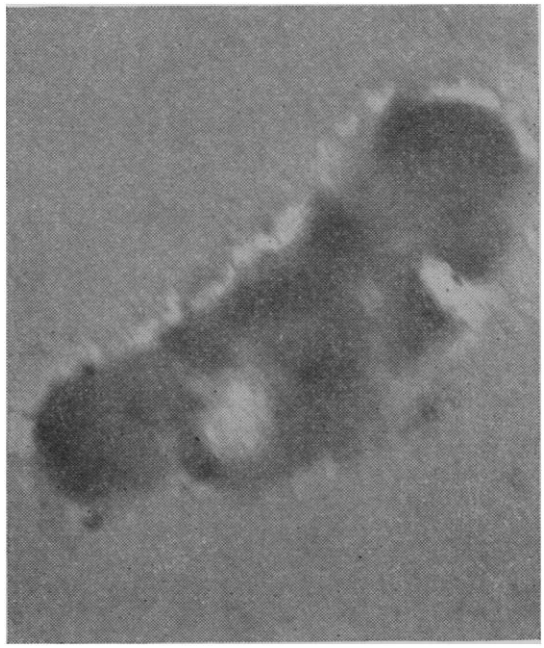

FIG. 5. X-ray-treated *E. coli* cell (electron microscope). Note irregular appearance.

normal cell. However, under the electron microscope, the elongated cells show characteristics not observed in the normal cell. As may be seen in Figs. 3, 4, and 5, some of these cells have a definitely mottled and irregular appearance, certain

areas being less dense than the remainder of the cell. In Fig. 6, which shows parts of the same cell, another unusual feature may be seen. Some of the elongated *E. coli* show definite "breaks" in the cell, perhaps at the place where the cell would have divided normally. Gates (*6*) reported that his irradiated

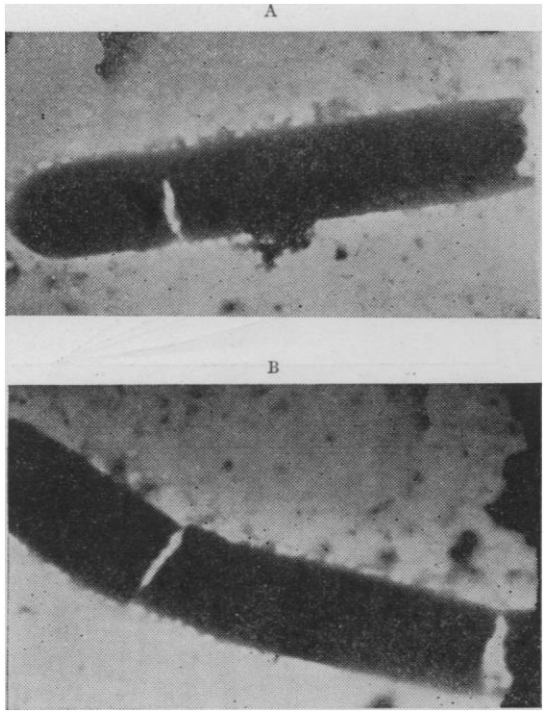

FIG. 6. A: X-ray-treated *E. coli* cell (electron microscope). Note periodic "break" in cell; B: part of same cell.

bacteria broke up into units, which then degenerated without further growth. Fig. 6 may be an indication of this type of behavior.

A possible explanation for "breaks" in the cell is that the enzyme which is responsible for the pinching off of the cell wall in normal bacterial fission has been destroyed by X-rays while other enzyme systems continue to function.

References

1. BEADLE, G. W. *Amer. Sci.*, 1946, **34**, 31-54.
2. CLARK, G. L., and BORUFF, C. S. *Science*, 1929, **70**, 74.
3. DEMEREC, M. *Proc. nat. Acad. Sci., Wash.*, 1946, **32**, 36-46.
4. DREA, W. F. *Amer. Rev. Tuberc.*, 1938, **38**, 205-228.
5. EULER, H., AHLSTROM, LUCIE, and HOGBERG, B. *Z. physiol. Chem.*, 1942, **277**, 1-17.
6. GATES, F. L. *Science*, 1933, **77**, 350.
7. GRAY, C. H., MARTON, L., and TATIUN, E. L. *J. appl. Phys.*, 1945, **16**, 265.
8. HABERMAN, S., and ELLSWORTH, L. D. *J. Bact.*, 1941, **40**, 483-503.
9. HOLLAENDER, A. *Annu. Rev. Physiol.*, 1946, **8**, 1-16.
10. KEMPSTER, C. *Lancet*, 1917, **11**, 423-424.
11. LEA, D., HAINES, R., and BRETCHER, E. *J. Hyg.*, 1941, **41**, 1-16.
12. LEA, D. E., HAINES, R. B., and COULSON, C. A. *Proc. roy. Soc.*, 1937, **123B**, 1-21.
13. LINCOLN, R. E., and GOWEN, J. W. *Genetics*, 1942, **27**, 441-462.
14. MULLER, H. J. *Science*, 1927, **66**, 84-87.
15. NEWCOMER, H. S. *J. exp. Med.*, 1917, **26**, 657-668.
16. RAHN, O. *Bact. Rev.*, 1945, **9**, 1-47.
17. WILKIN, E. M. *Proc. nat. Acad. Sci., Wash.*, 1946, **32**, 59-68.

A Mirror Device for Studying Lower Surfaces of Small Objects Using a Dissecting Microscope

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The problem of viewing all sides of a minute object such as a small flower, a small insect, and the like can often be not only difficult but exceedingly exasperating. The writer has for some time been studying a group of plants, the flowers of which are not over 3 mm. in size. A binocular microscope was necessary for accurate study of these. In order to count parts, observe arrangement, etc., it was necessary, after observing the upper surface, to turn the flower over painstakingly for a view of the lower surface. This method was very unsatisfactory because of time wasted, the possibility of losing the object, and the uncertainty arising from inability to see the entire object at the same time.

Recently, however, a solution to this problem has presented itself and is described here with the hope that it may be of use to other workers faced with similar problems. The device is quite simple and can be constructed in a few minutes by anyone. The model which has been used by the writer (Fig. 1)

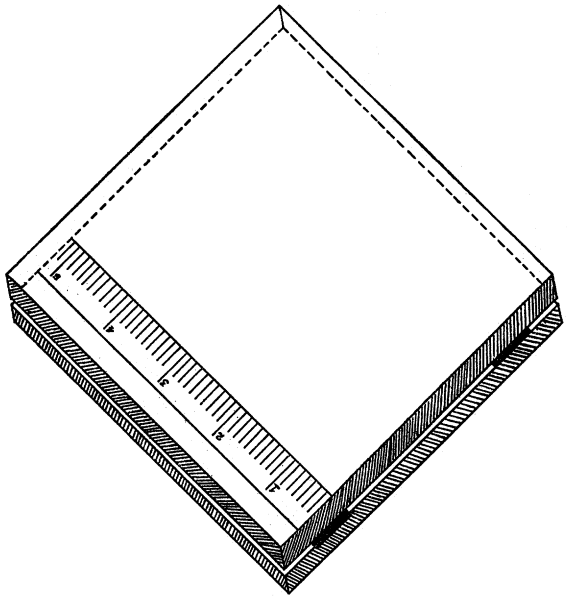

FIG. 1

consists of a piece of plate glass, $\frac{5}{8}$ inch thick, mounted on top of a small mirror. This is placed on the stage of a binocular dissecting microscope, and the object to be viewed is placed on top of the glass plate. The lower surface may be seen by merely focusing toward the object a distance equal to slightly less than twice the thickness of the glass used. This action brings the image of the lower surface distinctly into view, and the results are the same, regardless of the magnification used. In the present model a millimeter scale has been slipped between the mirror surface and the plate glass, but a scale,