duction, mutations of both lethal and visible types, sterility, and death, depending upon the time and technics of exposure. More tests were made with the CLB method, whereby an analysis was made of effects on the X chromosome in the sperm of the treated males. The effects observed in this test include lethal mutations, visible mutations affecting the wings, and inversions, as determined by cross-over tests. The frequent finding of unilateral wing mutations in the F_2's suggest that most CLB or other tests should be carried through the F_3 generation. Treated females carrying recessive genes show increased rates of primary nondisjunction of the sex chromosomes.

More detailed accounts of these investigations will be published elsewhere at a later time. The authors are indebted to S. I. Ward, president of the Crystal Research Laboratories of Hartford, Connecticut, for the loan of the ultrasonic equipment used in this research.

A Rapid Method of Single Cell Isolation

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During the course of an investigation on *Chlamydomonas* it became necessary to isolate particular zygotes. A simple, accurate method was developed, based on variations of the principles used by Edgerton (*2*), La Rue (*3*), and Dickinson (*1*). The present method requires the construction of two special instruments, a marking device and a supporting slide.

One part of the marking device was made from a sheet of Lucite 1.5 mm thick. A circular disc (E) was cut and ground to a diameter equal to the width of the flat lens surface of the Abbé condenser on a compound microscope. A small hole about 0.5 mm in diameter was then drilled in the exact center of the disc. The other part of the marking device consisted of a small-bore glass tube (D), the diameter of which was somewhat less than the width of the visual field of the compound microscope. Pyrex glass tubing with an outside diameter of 5.0 mm was drawn out to 0.5 mm diameter over a Bunsen burner and fractured so that a sharp, clean, 90° angle was obtained. The tip was then sharpened by rotating the fractured end of the glass tube between a carborundum stone and forefinger until a good cutting angle was obtained. After fracturing the glass tubing about 3.0 mm from the sharpened tip, the newly fractured end was glued into the hole, in the center of the Lucite disc (Fig. 1). The assembled piece was mounted on the lens of the Abbé condenser and the upthrust cutting edge centered in the visual field of the microscope. A drop of glycerin on the surface of the lens acted to stabilize the instrument and clarify the optical system. Ultraviolet light was used to sterilize the sharpened tip before it was used.

The supporting part of the apparatus (B) consisted of a piece of Lucite cut to the same dimensions as a standard glass microscope slide. From the center a 15.0-mm-square segment was removed.

A piece of sterile 4.0% agar (C) about 10.0 mm square and 0.5 mm thick is placed in the center of a cover slip 22.0 × 40.0 mm (A). The cells to be isolated are gently streaked with an inoculation needle onto the surface of the agar square. The cover slip with the inoculated agar square is inverted and centered in the window of the Lucite slide, a drop of glycerin on each end of the cover slip serving to anchor it to the slide. The whole mount is then placed on the stage of the microscope and the cells observed *through the agar* with the low-power objective (about 100 ×). Each cell may be studied under high power for further determination of its suitability for isolation. When a desirable cell is located, the condenser

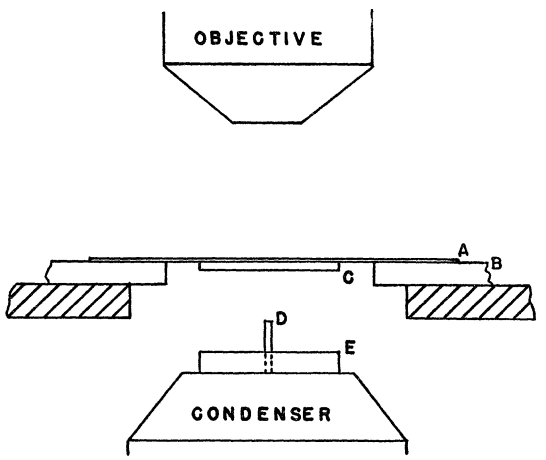

FIG. 1. Assembled isolation apparatus.

is raised until the shadow of the ascending glass tube appears in the center of the field of the low-power objective, the Lucite slide then being moved horizontally until the cell is approximately in the center of the circular shadow. The condenser with its marking device is slowly raised until the sharpened edge of the glass tube cuts a ring around the desired cell. The cutting edge is worked upward until it rests against the cover slip so that the agar plug and its cell are thus completely severed from the surrounding agar. The process of "ringing" cells may be repeated several times on the same piece of agar.

After the cells are marked, the cover slip with the agar square is placed, agar side up, on the stage of a binocular dissecting microscope, and the agar plug, together with the accompanying cell, is removed with a platinum-iridium microscalpel and placed on a suitable culture medium.

The method described is effective in the isolation of very small cells (about 2.0 μ). By this procedure 30 or more single cell isolations may be made in 1 hr. Also a particular cell may be studied in detail at a magnification up to 800 × prior to isolation. The apparatus is made from materials available in any biology laboratory.

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