The experimental mixture is in turn delivered from the metering jar to the secondary mixing chamber and accumulator (L) by the automatic siphon (K). K′ is an obvious device at the intake of the siphon to give a clean break of the flow of fluid at the end of a cycle. The principle of this device is gained by the air trap maintained in it as the level of fluid rises.

The accumulator (L) serves two purposes. As already indicated, it gives a secondary mixing and provides a constant flow of water to the experimental animal, thus eliminating the effect of an interrupted flow.

The tubes (M, M′) are siphon and overflow tubes, respectively, to maintain the uninterrupted flow of water from the accumulator to the animal chamber. In this particular case the constant level chamber (N), as developed by Galtsoff (*1*), Nelson, Loosanoff, and others, was used.

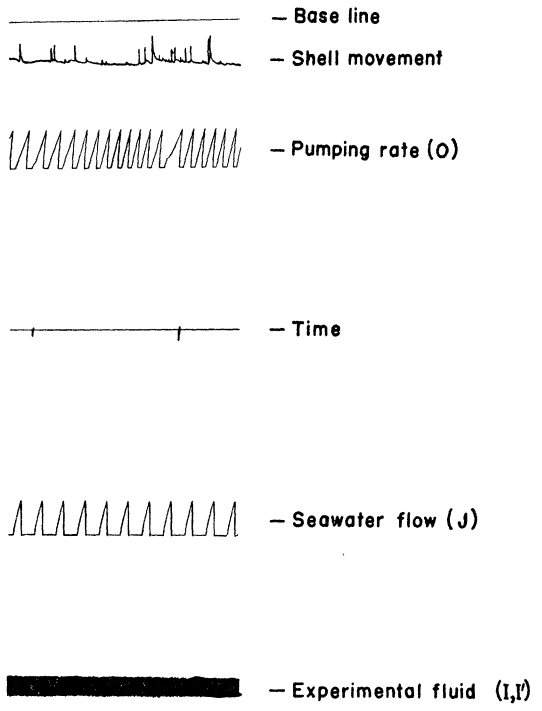

FIG. 2. Sample of record obtained by apparatus described. Letters in parenthesis are reference letters used in text.

In this respect our principal deviation from their developments is the method of measuring the water pumped by the oyster. This, in our case, is simply a receiving chamber (O) provided with an automatic siphon like that described for the metering jar above. The receiving chamber is also equipped with a hydrostatic tube and recording tambour, as above.

The principal criticism of this apparatus would be that the fluids flowing in while the siphons are emptying the respective vessels would not enter into the final aggregates recorded. This, however, is taken care of by calibrations derived from measurements of actual flow from the delivery ends of the siphons while they are in operation. The measuring box for the oyster chamber has the advantage of giving direct information on the minute changes in the pumping behavior of the oyster. This is illustrated in the sample record shown in Fig. 2.

Reference

1. GALTSOFF, PAUL S. Reaction of oysters to chlorination. (Research Rep. 11, U. S. Fish & Wildlife Service, Washington, D. C.)

The Induction of Cytogenetic Variations by Ultrasonic Waves

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Root tips of *Allium* and *Narcissus*, shoot tips of *Helianthus*, and young adults of *Drosophila melanogaster* were treated in an intense ultrasonic field generated by a piezoelectric instrument with an output of approximately 150 watts acoustic (by calorimetric determination) in the zone where the material was exposed. The vibration frequency used was 400,000/sec. Exposures were made in several types of specially constructed chambers. The technic of exposure, although not yet completely standardized, has yielded sufficiently promising results to warrant a preliminary report.

Helianthus plants now growing in the greenhouse after having their apical meristems treated in the seedling stage show definite phenotypic appearances suggestive of genetic changes which are corroborated by cytologic examination of treated root tip material. Some show a hypertrophy and a thickened, rugose condition of the leaves reminiscent of the results of colchicine treatment.

Chromosome examinations of root tip smears and sections show frequent breakage of whole chromosomes and individual chromatids. Late prophase, metaphase, and anaphase chromosomes show an almost complete uncoiling with the chromatids lying parallel, with numerous breaks, attenuations, fusion of parts, and other general evidences of physical disruption. Interphase nuclei often appear as though lysed and are sometimes extended the length of the cell in a spiral form. The nuclear membrane of such deformed nuclei in some cells is destroyed; in others it appears to be intact. In some cells the interphase nuclei, nucleoli, and the cytoplasm were completely segmented into two to four integral parts. Spindle figures of dividing nuclei in affected areas seem to be totally destroyed.

Despite the observed general disruption of the cell system, recovery as measured by resumption of growth seems to be general in all but those tissues which showed general collapse by the longer exposures. In collapsed tissues, no evidences of discrete or dispersed nuclei could be found by staining.

Young adult flies, etherized just prior to treatment, show effects ranging from none through phenocopy in-

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

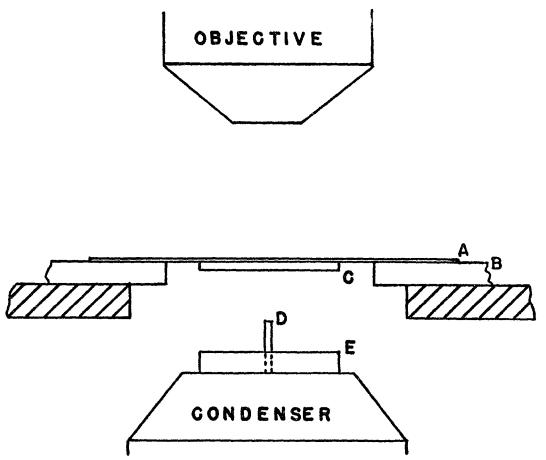

FIG. 1. Assembled isolation apparatus.

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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2. EDGERTON, C. W. *Phytopathology,* 1914, **4**, 115.
3. LA RUE, C. D. *Bot. Gaz.,* 1920, **70**, 319.