IN THE LABORATORY

An Automatic Proportioning Apparatus for Experimental Study of the Effects of Chemical Solutions on Aquatic Animals[1]

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In carrying out a considerable amount of experimental work in connection with investigations on the effects of industrial pollution on oysters it was found desirable to have an apparatus for the injection of experimental fluids in constant proportions. The requirements of such an apparatus would be: (1) flexibility of proportioning, automatic change of injection with changing flow of water from laboratory sea water supply, and operation over long, uninterrupted periods of time; (2) a minimum of metal surfaces in contact with fluids; (3) dependability and ease of maintenance; (4) adaptability to kymographic recording; and (5) low cost. Also, it would be highly desirable to keep a given oyster under the influence of the experimental fluids as long as possible, with a minimum of fatigue due to the experimental environment. The apparatus to be described here (Fig. 1) fulfilled all of these conditions better than any the authors were able to find described in the scientific literature (*1*) or in the catalogues of manufacturers of commercial proportioning apparatus.

The mechanism in general is a proportioning apparatus activated by the inflow and outflow of water from the sea water supply. This activation occurs by means of a float which operates a ratchet mechanism which in turn lowers a siphon connected to the reservoir containing the experimental fluid. By this arrangement an automatic proportioning is obtained. Each time the float rises and falls a definite amount of experimental substance is delivered from the reservoir, whether or not the float rises and falls fast or slow.

The sequence of events in the operation of the mechanism can be related as follows: The activating float (A) rises with the inflow of water into the metering jar (B). As this happens, the lever (C) lowers and turns the ratchet wheel (D) in a counterclockwise direction. This unwraps the string from the spool (E) and so lowers the block carrying the outlet (F) of the siphon (G) which drains fluid from the reservoir (H). The amount of fluid delivered is dependent entirely on the distance traveled by F on each cycle of the float and the diameter of the reservoir. The distance traveled by F on each cycle can be varied by changes in the diameter of the spool (E) and by the number and size of teeth in the sprocket wheel (D). The drops from F fall into the splashproof cone

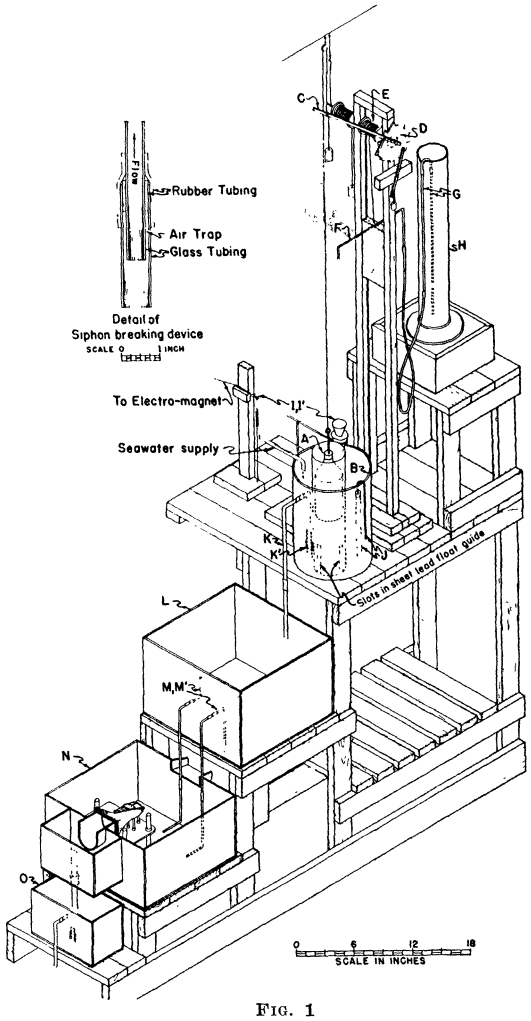

FIG. 1

drop indicator (I, I′) and thence to the metering jar as it fills and where the first mixing occurs. Because of the turbulence within the jar, this mixing is quite thorough. The indicator is wired to an electromagnet which records the inflow of experimental fluid on the kymograph record. The hydrostatic tube (J) is connected to an ordinary recording tambour and transmits the rate of flow of sea water into the metering jar to a recording on the kymograph. Thus, an automatic record is obtained of both the introduction of the experimental fluid and the rate of flow of sea water.

[1] This apparatus was developed by the authors while using space and facilities kindly loaned to them by the U. S. Fish and Wildlife Service at Pensacola, Florida, for the purpose of carrying out independent investigations.

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.

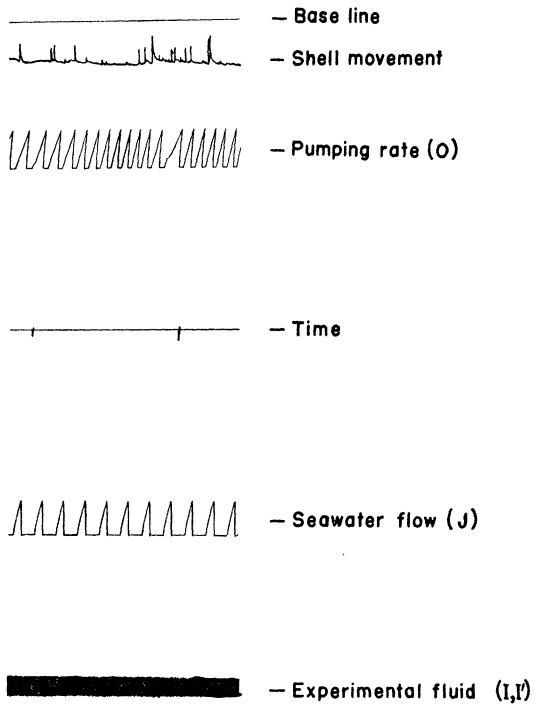

FIG. 2. Sample of record obtained by apparatus described. Letters in parenthesis are reference letters used in text.

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-