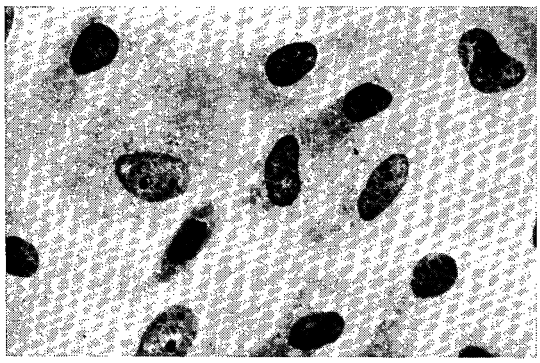

FIG. 2. Microscopic detail of portion of culture prepared by the method described. Rat embryo spleen, fixed in Carnoy's fluid, Leishman stain (×600).

solution does not have to penetrate a wet gel-like coagulum.

The mounted preparations are thin and easily visualized under the high-power objectives of the microscope. They dry completely in 24–48 hrs in an incubator and can be filed upright. The dense, heavily stained background of the usual total culture mount is either absent or considerably diminished. The cells stain evenly and sharply and show no more distortion of structure or shrinkage than cells fixed and prepared by any other method.

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Removal of the Fertilization Membranes From Large Quantities of Sea-Urchin Eggs

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In the course of developing a method for separating on a large scale the meso-, macro-, and micromeres of the sea-urchin 16-cell stage we have had, first of all to devise a means of removing the fertilization membranes. This is usually done on a small scale by sucking the eggs through a fine capillary pipette, with a diameter just large enough to allow the eggs, but not the membranes, to pass. In this way, some hundreds of eggs can be dealt with quite quickly. Multiplying the fine opening of the pipette many times by using a sieve of bolting silk, as proposed by Just (*1*), we have removed the membranes from large quantities of eggs in a matter of seconds. This method, which may perhaps be of interest to others working with sea-urchin eggs, is described briefly below.

With a sieve of this kind, three factors are important: (1) the exact moment for treating the eggs, (2) the mesh of the bolting silk, (3) the rate at which the eggs are passed through the silk. Since fully elevated membranes are most easily torn off and since the membranes harden rather soon after elevation, the time interval for treatment is limited. With *Paracentrotus lividus* we have found, as have others, that the best moment for removing the membranes is about 30 sec after the beginning of elevation. For the eggs of this species, with a diameter of about 90 μ at Roscoff, we have found bolting silk No. 13xxx (70 × 70 μ) to be suitable. If an egg suspension is allowed to flow by its own weight through this mesh of bolting silk, the membranes pass unbroken. If, however, a water suction pump is used to draw the suspension through the silk at a high speed, all the membranes are removed, but many eggs are broken. To draw

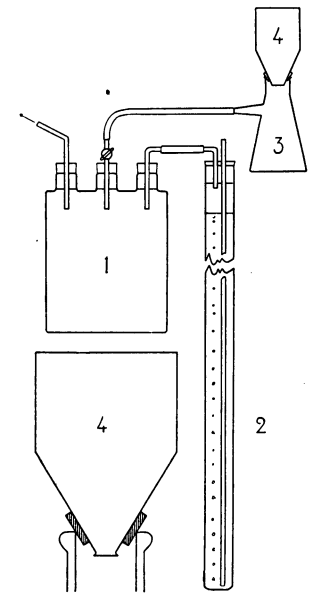

FIG. 1

the eggs through at the right speed we have used the arrangement shown in Fig. 1. A 2,000-cc Wolf flask (1), with three openings, is connected to a water suction-pump, to a water-column pressure regulator (2), and, via a tap, to a small suction flask (100–250 cc) (3). The container for the egg suspension (4), which has an opening at the bottom (diameter, 18 mm), covered with bolting silk, sits in a rubber packing on this suction flask.

Before use, the pressure regulator is filled in order to give a negative pressure of 800–1,000 mm of water (the denser the egg suspension, the greater the pressure needed). The tap is closed and the suction pump is turned on, thus lowering the pressure in the Wolf flask, until the regulator allows air to come in. The eggs are then inseminated, and the suspension is transferred to the container just before the membranes are fully elevated. The tap is opened, and the suspension is sucked into the

flask. In this way, the fertilization membranes of 98–100% of the eggs can be removed.

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Electrophrenic Respiration

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A new type of artificial respiration has been developed which uses electrical stimulation of one or both phrenic nerves. Current is supplied by a Grass stimulator set to deliver 40 impulses/sec, each impulse having a duration of 2 millisec. The current is fed through a rotating potentiometer which describes an arc, the length of which can be set by an adjustable lever. This lever is driven at a rate that can be adjusted by a friction clutch arrangement. In this way, the voltage delivered to the stimulating electrode is rhythmically raised and lowered at that rate per minute at which it is desired to maintain respiration. The increase in voltage is gradual and results in a smooth diaphragmatic contraction that produces effective inspiration. The reverse permits effective expiration by the passive relaxation of the diaphragm.

Within satisfactorily flexible limits, an increase in peak voltage applied to the nerve results in an increased force of diaphragmatic contraction and an increased tidal and minute volume. A linear relationship has been shown to exist between peak voltage applied and the minute volume accomplished, making easy the adjustment of the effective depth of respiration.

Electrophrenic respiration has been studied in the rabbit, cat, dog, monkey, and man. From the obtained data the following observations have been made:

(1) A smooth diaphragmatic contraction, closely resembling that seen during spontaneous respiration, follows the application of a gradually increasing voltage applied to the phrenic nerve.

(2) The ventilation accomplished by stimulating the phrenic nerve is, within satisfactory limits, directly proportional to the peak voltage applied.

(3) The spontaneous minute volume, arterial blood oxygen and carbon dioxide tensions of the experimental animal and man can be duplicated by the use of electrophrenic respiration with the submaximal stimulation of one phrenic nerve in the absence of spontaneous respiratory activity.

(4) Maximal stimulation of one phrenic nerve can produce overventilation and alkalosis. Bilateral maximal phrenic nerve stimulation produces a further decrease in arterial blood carbon dioxide tension and may triple the animal's spontaneous minute volume. These facts indicate the reverse of the method.

(5) An adequate minute volume and normal arterial blood oxygen and carbon dioxide tensions have been maintained in the cat for as long as 22 hrs in the absence of spontaneous respiratory activity by electrophrenic respiration.

(6) The experimental animal under anesthesia and unanesthetized man promptly relinquish control of respiration when adequate electrophrenic respiration starts.

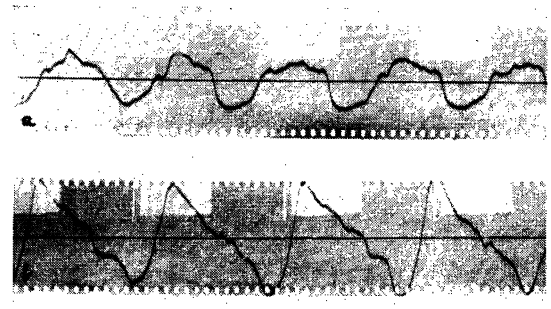

FIG. 1. Pneumotachogram of 33-year-old female. The area within the curve under the base line is directly proportional to the amount of inspired air. The area above the base line represents expiration.

Fig. 1 shows the pneumotachographic tracing obtained from a 33-year-old woman (a) during spontaneous respiration and (b) during electrophrenic respiration. The apparatus used was that described by Silverman (*4*) and by Silverman and Whittenberger (*5*). It can be seen that with a rate slightly slower than the patient's spontaneous rate, electrophrenic respiration produced a larger tidal and minute volume than that produced by spontaneous respiration.

In the same patient, studies of arterial blood oxygenation with the Millikan oximeter (*1*) revealed that arterial saturation could be increased over spontaneous levels by means of electrophrenic respiration.

Since the preparation of this manuscript, an opportunity arose for using this method of artificial respiration on a 5-year-old boy with complete respiratory paralysis following rupture of a cerebral aneurysm. As the only means of artificial respiration, it was capable of sustaining life for 52 hrs. Additional data are in press concerning the blood gas tensions and minute volumes achieved with this technique (*2*), its use in man (*6*), and the mechanism of suppression of spontaneous respiratory activity during electrophrenic respiration (*3*).

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