amount administered was adjusted in order to maintain the original dosage. At the end of four weeks they were again weighed. The table shows the percentage increase in weight for the four-week period.

The differences between the control group and those given dosages of 0.28, 2.80, and 28.00 mg/kg were not significant at the 5% level. The difference between the control group and the group given a dosage of 280 mg/kg was barely significant at the 5% level.

Next, experiments were started to determine the lethal dose of the alkanolamine of 2,4-D when diluted 1:9 with water. Each chick of a group of 5 (each averaging 166 gm) was given one dose of 380 mg/kg of body weight. These chicks survived.

Each chick of another group of 5 chicks (average weight, 111 gm) was given a dose of 765 mg/kg of body weight. All of these chicks died. Postmortem examination revealed a fatty degeneration with a pale mottling of the liver, spleen, kidneys, and heart. Hemorrhagic gastroenteritis was also evident.[2] Hence, for small chicks the lethal dose of diluted 2,4-D is somewhere between 380 and 765 mg/kg.

The fact that a single dose of 765 mg killed, but not a total dose of 3,360 mg administered over a four-week period (280 mg/kg chicks of Table 1), indicates that the alkanolamine of 2,4-D is not a cumulative poison.

The question might be raised as to the possibility of chicks being killed by feeding on plants which had been sprayed with 2,4-D. At a spraying rate of 1 lb of 2,4-D/acre, a chicken weighing 1 kg would have to consume *all* of the 2,4-D applied on 72 sq ft within a day or two to obtain a lethal dose.

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A New Histological Procedure for Whole Tissue Cultures Grown in Plasma

ANNABELLE COHEN and CHARITY WAYMOUTH

Chester Beatty Research Institute, Royal Cancer Hospital, Fulham Road, London, S.W.3

Standard textbook procedures (*2, 3*) recommended for fixing and staining whole tissue cultures in plasma coagula have proven unsatisfactory for the following reasons: (1) The dense fixed coagulum presents a nearly impermeable barrier to the stain, thus necessitating prolonged staining periods; (2) the coagulum itself stains diffusely and heavily, preventing adequate contrast between the cells and the medium and necessitating careful and time-consuming decolorizing and developing procedures; (3) the resulting preparation, when mounted, is thick, has a tendency to form air bubbles, does not dry readily, and is generally inadequate for microscopic study. Tompkins, Cunningham, and Kirk (*4*) recently attempted to improve results by washing cultures in a salt solution for 4 hrs at 7° C to remove soluble protein before fixation, but still had to resort to prolonged staining (1 hr in Delafield's hematoxylin) followed by several hours of washing. Earle (*1*) has devised complicated fixing, staining, and mounting procedures, too elaborate to warrant evaluation here.

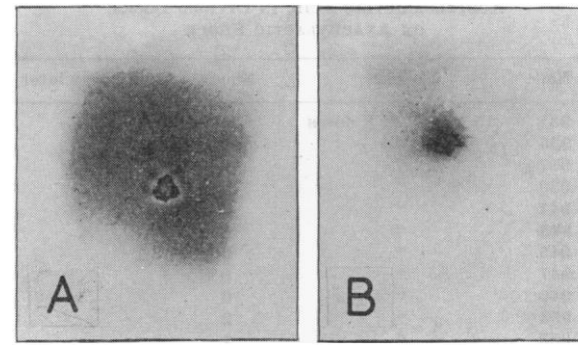

FIG. 1. Gross appearance of stained tissue cultures: A, plasma undried; B, plasma dried (×1.5).

We have found that most of the disadvantages of the plasma coagulum can be eliminated by drying the whole tissue culture preparation after fixation. The procedure is as follows: The culture in its coagulum on a cover slip is fixed overnight in 3–4% formol containing 0.5% acetic acid or in Carnoy's fluid for 1 hr. In general, acetic-alcohol fixations give a more granular and more opaque dried specimen than acetic-formol fixations. The cover slip is then washed *thoroughly* in running water (after acetic-formol) or in descending strengths of alcohol, and water (after Carnoy's). After a final rinse in distilled water the cover slips are laid flat on a glass surface protected from dust (Petri dishes or staining dishes can be used) and allowed to dry slowly and thoroughly in air. If a preparation of a tube or a flask culture in plasma is desired, the portion should be fixed and washed *in situ*, pried loose and placed, like a paraffin section, in a dish of water, where it can be manipulated with a dissecting needle onto a slide or cover slip previously coated with a thin film of Mayer's albumin, and dried as above. Cultures grown in a fluid medium should not be dried. The dried cultures may be stained with any one of the hematoxylins for from 5 to 15 min, depending on the strength of the stain. Weigert's iron-hematoxylin has been found very satisfactory. The staining is simple to control after a preliminary trial, and prolonged washing to develop the color is not necessary. Once stained, the cultures should not be allowed to dry again. Following this, the cover slips are passed through the alcohols in the usual manner, cleared in xylene, and mounted. If a Leishman or Giemsa stain is used, acetone and acetone-xylene dehydrations should be employed instead of the alcohols. The dehydration and clearing procedures take considerably less time with dried cultures, since each

[2] The authors are grateful to Robert W. Lindenstruth, who made the examinations.

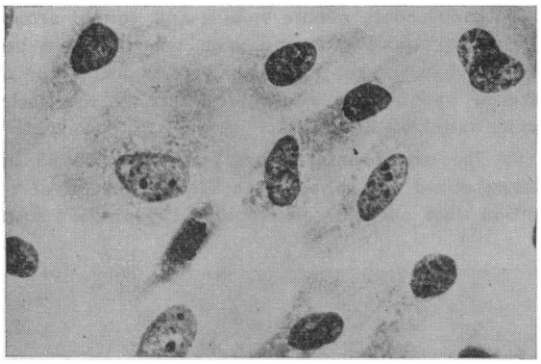

FIG. 2. Microscopic detail of portion of culture prepared by the method described. Rat embryo spleen, fixed in Carnoy's fluid, Leishman stain (×600).

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

P. E. LINDAHL and J. LUNDIN

Institute of Zoofysiology, University of Uppsala, and *Station Biologique, Roscoff*

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

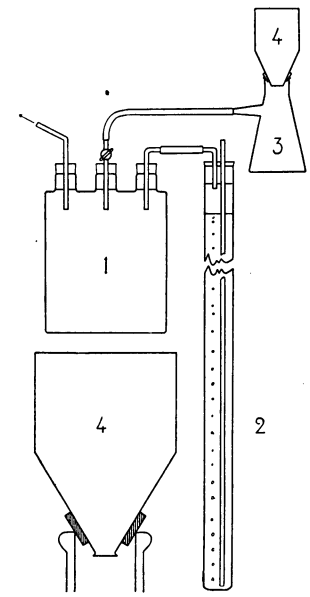

FIG. 1

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