Summary.—An apparatus providing continuous artificial illumination of constant intensity directed to the lower surface of flounders is pictured. Pigmentation was developed on the lower normally unpigmented surface in a high percentage of summer flounders in the following experimental situations: (1) Unoperated fishes in black tanks illuminated from below. (2) Blinded dark fishes in black tanks illuminated from below or in white tanks illuminated from above.

The observation that flounders blinded in the dark phase developed ventral pigment as readily as unoperated ones indicates that the eyes are not essential to this reaction.

Light is a necessary factor in the production of ventral pigment.

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A RESPIRATORY PIGMENT FROM THE EGGS OF A MARINE WORM

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Of the wide diversity of pigments occurring in nature, a certain number are considered to function as respiratory carriers by virtue of their ability to be reversibly oxidized and reduced (see review of Barron[2]). I wish to report here the presence of such a pigment in the eggs of the marine worm *Urechis caupo*, together with evidence for its probable participation in cellular respiration.

The eggs of *Urechis caupo* are typically pink in color. In small, or relatively unripe, females, however, it is frequently found that the eggs are not pink, but yellow. Although the eggs of any one individual are all of the same color, a comparison of the eggs from different individuals shows

an intergradation of color from light yellow to an intense pink. It was found that if yellow eggs are placed in a Thunberg tube, and the tube then evacuated, they gradually become pink. If the vacuum is then broken and the eggs aerated, they regain their yellow color. The development of a pink color is apparently the result of the reversible reduction, *in vivo*, of a pigment present in yellow and pink eggs alike. The pigment in pink eggs can be oxidized by adding a small amount of H_2O_2 to the suspension (plus a trace of HCN to inhibit the powerful catalase contained in the cells). They thereupon become yellow. If the eggs are then washed and placed in an evacuated Thunberg tube they become pink again.

To obtain the pigment from the cells, pink eggs are extracted with acetone for 3–6 hours in a Soxhlet apparatus. This removes large quantities of two yellow pigments—one water-soluble and the other fat-soluble. The pink pigment remains behind. The nature of the two acetone-extractable pigments is as yet uncertain. Neither of them, however, shows reduction to a pink form. The pink pigment is then extracted by shaking with 5% HCl-methanol at 40°C. The pigment thus extracted consists of a mixture of the reduced form and its yellow oxidation product. If the extract is placed in the icebox overnight, the reduced form largely precipitates out in dark red, amorphous particles. The supernatant, containing the oxidized pigment and a small amount of the reduced pigment, is concentrated by distillation *in vacuo* and is finally dried on a water bath.

The oxidized form of the pigment is readily soluble in water. It is reduced by hydrosulfite, or by hydrogen in the presence of a platinum catalyst, to a pink (in concentrated solution, red) pigment. Upon shaking with air it reoxidizes to the yellow form. Autoxidation in air occurs rapidly at neutral and alkaline pH's. The reduced form is only sparingly soluble in acid solution ($<$pH 5.5). It is readily soluble at neutral and alkaline pH's, but immediately autoxidizes if oxygen is present. The pigment is rapidly destroyed by strong alkali. Autoxidation of the pigment can be accelerated *in vivo* by raising the intracellular pH by means of the penetrating base ammonia. Pink eggs so treated become yellow. Upon washing away the ammonia, the pink color returns.

The oxidation-reduction potential of the pigment has been determined polarographically[3] through the coöperation of Professor J. Percy Baumberger, using the purest preparation so far obtained. At pH 7.39 $E_0' = +0.163$ volt (250°C.). E_0' decreases 0.059 volt per unit increase in pH in the pH range 5–10. The change in E_h with change in degree of oxidation corresponds to a one-electron process. These results will be presented in detail in a future communication.

The facts that the pigment occurs naturally in both oxidized and reduced states, and that it is reducible by the cells and autoxidizes in the physiological range of pH, indicate that it is probably involved in the

cellular respiration. It is suggested that the pigment be called *urechrome* (not to be confused with the urinary pigment, urochrome). The chemical nature, absorption spectrum and physiological function of the substance are being studied.

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[2] Barron, E. S. G., *Physiol. Rev.*, **19**, 184–239 (1939).

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*FURTHER STUDIES ON THE PARTHENOGENETIC ACTIVATION OF RABBIT EGGS**

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In the course of certain studies on the artificial activation of rabbit tubal ova *in vitro* in which the development in culture of ova given certain stimulating treatments was contrasted with the development of untreated ova, we noted that in certain of the control cultures some ova gave clear evidence of activation. Our experiments were being conducted in a basement room at temperatures ranging between 17°C. and 21°C. Furthermore a period of one to two hours often elapsed between the sacrifice of the donor of the ova and their final incubation at 37.5°C. It seemed possible, therefore, that the cooling of rabbit ova might lead to activation. Some 80 unfertilized ova were cooled either by keeping them at room temperature for 2 to $3^1/_2$ hours (34 eggs) or by placing them in a refrigerator (at 6°C.) for 15 to 85 minutes (46 eggs). These ova, like all the others in our experimental series (see table 1), were cultured for 20 to 24 hours in rabbit serum at 37.5° (see Shapiro[3]), then fixed in Bouin's fluid (Pincus[1]), sectioned and stained with Ehrlich's hematoxylin, and examined for cytological evidences of activation.

In table 1 we present a summary of our data on the effects of cooling and also on the effects of exposing ova to: (1) balanced salt solutions made hypotonic by dilution with glass distilled water (usually 1 part salt solution to 1 part distilled water), (2) rabbit serum diluted to one-half by distilled water, (3) hypertonic balanced salt solutions (1.6 to 1.8% salt) and (4) hypertonic and hypotonic solutions alternately. Ova are considered activated when they exhibit clear pronuclei, or cleavage chromosomes, or cleavage. Ova classified as not activated either showed marginal meiotic