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.

[1] National Research Council Fellow in Zoölogy.

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

[3] Müller, O. H., and Baumberger, J. P., *Trans. Electrochem. Soc.*, **71**, 169–194 (1937).

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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

chromosomes (as at ovulation), or subnuclei (due to the scattering of meiotic chromosomes and nucleus reformation about single chromosomes or groups), or cytoplasmic fragmentation without cleavage. In some instances ova showed true cleavage had occurred followed or accompanied by cytoplasmic fragmentation—such eggs were considered activated.

The cooled ova appear to have been activated (and proceeded to cleavage) in a larger proportion than the other ova of these series. Actually our data indicate that cooling at 6°C. for 10 to 30 minutes was most effective: of 33 such ova 13 were activated and 10 cleaved, 7 of these cleavages without any cytoplasmic fragmentation. Of 13 ova placed at 6°C. for 85 minutes, 6 cleaved but 5 of these 6 showed cytoplasmic fragmentation.

We decided to attempt to cool ova *in situ* in the fallopian tubes. At 14 to 19 hours after the injection of an ovulating pituitary extract (Pincus[1, 2])

TABLE 1

THE EFFECTS OF VARIOUS TREATMENTS ON UNFERTILIZED RABBIT OVA CULTURED *in vitro* FOR 20 TO 24 HOURS

TREATMENT	NUMBER OF EGGS	NUMBER ACTIVATED	NUMBER CLEAVED	% ACTIVATED	% CLEAVED
Controls, no treatment	143	20	11	14.0	7.9
Cooling	80	42	19	52.5	23.8
Hypotonic balanced salt solutions	92	37	16	29.3	17.4
Hypotonic serum	354	134	32	37.9	9.0
Hypertonic balanced salt solutions	24	7	2	29.2	8.3
Alternating hypertonic and hypotonic solutions	29	9	2	31.0	7.0

the ovulated ova are massed below the first loop of the fallopian tube in a narrow portion and ordinarily so distend the tube as to be visible as a translucent bulge. We designed a hollow brass jacket which would enclose 3 centimeters of the tube at this point. Laparotomy was performed under combined ether and nembutal anesthesia, the sterilized cooling jacket placed about the right fallopian tube, and ice water circulated through the cooling jacket for appropriate periods of time.

Four females whose right fallopian tubes were so cooled for 15 minutes were sacrificed at various times after the operation. One killed on the second day after cooling showed a few ovulation points on each ovary, but only one uncleaved ovum was recovered from the cooled tube, none from the left (uncooled side). A second was killed at five days after cooling. Eight ova were recovered from the right oviducts of which one was a collapsed blastocyst, one a morula and the others fragmented or uncleaved. Two others sacrificed at 20 and 21 days, respectively, after the cooling operation

had neither eggs nor embryos, but one had a resorption site in the right uterus.

A number of other animals underwent the cooling operation and were allowed to go to term. The details are given in table 2.

TABLE 2

THE EFFECTS OF COOLING THE RIGHT FALLOPIAN TUBE CONTAINING FRESHLY OVULATED OVA

NUMBER OF RABBITS	PERIOD OF COOLING (MINUTES)	RESULT
1	5	No young
2	10	No young
7	15	No young
2	20	One gave birth to one living female
4	Frozen with solid CO_2 2 to 10 minutes	No young

Since these rabbits should have ovulated 12 to 15 ova on the operated side (Pincus[2]) it can be seen that one egg in over 200 developed into a living rabbit. This is less than would be expected if all the ova that presumably cleaved proceeded to develop normally. It has already been shown (Pincus[1]) that rabbit ova artificially activated by other methods may degenerate at any stage of development, and that the expectation of recovery of young is therefore very small.

Full details of these experiments will be published elsewhere.

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[1] Pincus, G., *Jour. Exp. Zool.*, **82**, 85 (1939).

[2] Pincus, G., *Anat. Rec.* (in press).

[3] Shapiro, H., *Science*, **90**, 308 (1939).