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Parthenogenetic Stimulation of Aged Anuran Eggs.

ANITA ZORZOLI AND ROBERTS RUGH.

From the Department of Biology, Washington Square College, New York University.

If eggs are retained in the uterus of the frog over an extended period so that insemination is delayed, it is known that radical changes take place in the cortex of such eggs¹ and very likely also in the second polar body elimination.² Since the region of activation is the cortex and survival of parthenogenetically stimulated eggs up to and beyond the tadpole stage may be dependent upon the diploid condition (polar body retention?),³ a significant difference in parthenogenetic data from aged eggs should be noted. A total number of 1807 eggs of progressively increasing ages were stimulated artificially and the incidence of cleavage and development compared with that of a similar number of control eggs which were less than one day old.

Hibernating females (*Rana pipiens*) were induced to ovulate⁴ and then kept in a refrigerator at 10°C for the duration of the experiment. On the 1st, 3rd, 5th, 7th, and 8th days eggs were stripped in 2 single rows onto glass microscope slides, pricked in the animal hemisphere with a fine glass needle and allowed to develop in spring water at 17°C. Seven hours after stimulation the eggs were examined and classified as showing surface furrows, deep furrows, and abnormal or normal cleavage. Controls consisted of normally inseminated aged and fresh eggs and pricked fresh eggs.

The data for 7- and 8-day-old eggs only and control 1-day-eggs are given in Table I. It is obvious that not only is there an increase in the total number of eggs showing deep furrows and abnormal cleavage but also a definite increase in the amount of normal cleavage. This difference between aged and fresh eggs disappears at the time of gastrulation. In fact, relative to the number of eggs initially undergoing normal cleavage, there is a smaller percentage of aged eggs which survive gastrulation.

As mentioned before, aging radically affects the integrity of the egg cortex. Zimmerman and Rugh¹ observed changes in permea-

¹ Zimmerman, L., and Rugh, R., *J. Morph.*, 1941, **68**, 329.

² Bataillon, E., and Tehou Su, *C. R. Acad. Sci.*, 1932, **195**, 353.

³ Parmenter, C. L., *J. Exp. Zool.*, 1933, **66**, 409.

⁴ Rugh, R., *Biol. Bull.*, 1934, **66**, 22.

TABLE I.

Frog	Age of eggs	No. of eggs	% surface furrows	% deep furrows	% abnorm. cleav.	% normal cleav.	% gastrulating	% hatching
Control	1	267	20	4	0.8	1.6	.1	.0
A ₁	7	211	42	52	1.4	2.8	1.8	1.4
A ₂	7	218	44	50	4.6	3.2	0.2	.0
A ₃	7	232	10	70	9.0	9.5	0.9	.3
Control	1	260	17	18	0.8	1.9	1.6	.1
A ₁	8	30	43	53	0.0	3.3	0.0	.0
A ₂	8	84	37	56	3.6	2.8	0.0	.0
A ₃	8	100	25	59	5.0	8.0	2.0	.0

Abnormal cleavage—cell stages recognizable but cells not in normal relationship.
 Normal cleavage—normal cell relationships and size.

bility, pigment distribution and cytoplasmic vacuolation with increasing age of *Rana pipiens* eggs. To account for the higher incidence of abnormal and normal cleavage in this experiment it might be conjectured that these cortical changes are responsible for a greater sensitivity to stimulation and resultant cleavage. In this connection it is interesting to note that after normal insemination of 7- and 8-day-old eggs, a high percent of the eggs failing to undergo development showed surface and deep furrowing similar to and in some cases identical with that found in the parthenogenetically stimulated eggs. However, as the eggs reach the critical time of gastrulation the derangement of cytoplasmic materials may be sufficient to impede the extensive movements of materials which normally occur. Embryos which went through this stage often showed signs of imperfect gastrulation such as: microcephaly and persistent yolk plug. On the other hand, some cases did develop normally beyond gastrulation. It might be that these resulted from eggs which retained the second polar body as a consequence of aging. It is evident that polar body and early cleavage history studies as well as chromosome counts must be made for aged eggs as were made by Parmenter⁵ for normal eggs.

Summary. Aged eggs of *Rana pipiens*, parthenogenetically stimulated, showed a higher percentage of cleavage but a proportionately lower incidence of gastrulation than normal eggs similarly treated.

⁵ Parmenter, C. L., *J. Morph.*, 1940, **66**, 241.