

INDUCED OVULATION IN *RANA PIPPIENS* III

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The relation of the pituitary gland to ovulation in amphibians has been studied since the work of Wolf in 1929. Implantations of the pituitary into the lateral or femoral lymph sac¹ and under the skin², and injections of pituitary suspensions into the abdominal vein,² dorsal lymph sac or hind leg muscles,³ and body cavity,^{4, 5, 6} have all caused ovulation. Injections of other substances besides amphibian pituitaries such as human placenta,⁷ whole sheep pituitary,⁴ antuitrin-S,⁸ human pregnancy urine,^{7, 8} progesterone,⁹ testosterone and methyl testosterone,¹⁰ have also induced more or less ovulation. These studies on the effect of various substances on the ovulation process have been complicated by the fact that the ovaries were left in the animals during the period of ovulation. A quantitative measurement of the amount of ovulation is difficult to obtain under such circumstances. The writer, in collaboration with Henry C. Hill, Jr., has shown^{11, 12} that ovulation may be studied quantitatively by removing the ovaries some hours after injection of the frog and suspending them in Ringer's solution.

Recently ovulation has been obtained from ovaries which were removed from normal uninjected females and placed in Ringer's solution containing a suspension of female pituitaries. Zwarenstein¹³ reported that ovulation can be induced in excised ovaries of *Xenopus*, suspended in Ringer's solution, by the addition of progesterone, and Shapiro and Zwarenstein¹⁴ showed that testosterone and androstenedione are similarly effective. Experiments reported in this paper prove that ovaries from *Rana pipiens* will ovulate when placed in Ringer's solution containing *Rana pipiens* pituitaries.

Method.—Hibernating frogs received from Wisconsin were soaked in cold water and placed in a refrigerator at 0 to 1° C. until used. They were periodically removed, bathed in cold water, and returned to refrigerator. Under these conditions good ovulation was obtained in

experiments covering a month's time. Pituitary glands were removed from females, placed in Ringer's solution, and broken up into a fine suspension. Various concentrations of the hormones for each experiment were prepared by dilution from such a pituitary suspension. Ovaries from the same females used for the pituitary suspension or from other females were removed and portions of these ovaries were suspended by Nichrome wires in vials containing the Ringer-pituitary suspension. Ovulation occurred in these pituitary suspensions, and by periodic counts until ovulation ceased the rate and total amount of ovulation was determined.

Experiments

1. *Effect of pituitary concentration on ovulation.*

Six experiments were performed in which the ovaries were placed in various concentrations of female pituitaries, ranging from zero to one pituitary per ml of suspension. The results of these experiments are summarized in Table I.

Only one egg was obtained in six control vials which contained the Ringer's solution but no pituitary. No eggs were found in the six vials which contained 0.001, 0.002, and 0.005 pituitary per ml. Starting with 0.01 some ovulation occurred, increased to a maximum around 0.08 and decreased to a minimum at 1.0 pituitary per ml. Experiments performed a year ago, using concentrations of several pituitaries per ml, gave no ovulation. The occurrence of a maximum effect is interesting because it shows that some inhibitory factor, which increases with concentration, must be present; and the low optimal concentration (0.08) may indicate something of the amount of pituitary normally needed in the female at the time of ovulation.

2. *Effect of methods of storage of the frogs on the subsequent ovulation.*

In a second series of experiments a new shipment of female frogs was divided

TABLE I.—EFFECT OF PITUITARY CONCENTRATION ON OVULATION CONCENTRATION IN PITUITARIES PER ML OF RINGER-PITUITARY SUSPENSION

	0	.001	.002	.005	.01	.02	.03	.04	.05	.06	.07	.08	.09	.10	.20	.50	1.0
Average eggs per vial.....	0	0	0	0	12	11	23	26	15	9	25	28	13	12	8	5	5

TABLE II.—EFFECT OF TWO METHODS OF STORAGE OF FROGS ON OVULATION JAR A FROGS KEPT IN WATER; JAR B FROGS IN MOIST AIR

	Pituitaries from		Ovaries from		Pituitaries and ovaries from	
	Jar A	Jar B	Jar A	Jar B	Jar A	Jar B
Average eggs per vial.....	26	28	39	15	35	12

in two groups. One group of frogs was placed in a large jar (A) containing about one inch of water, the other group in a similar jar (B) containing cheese cloth saturated with water. Thus, some of the frogs (jar A) were continually in water and the others were always in a moist atmosphere, but not actually in water. Both jars of frogs were kept in the refrigerator at 0 to 1° C. Four combinations of pituitary suspensions and ovaries were made. Ovaries from jar A frogs were tested in suspensions of pituitaries from both jar A and jar B frogs and similarly for the ovaries of jar B frogs. In all cases the suspensions were made up to a concentration of 0.08 pituitary per ml. The results are summarized in Table II.

The pituitaries of both sets of frogs were equally potent when tested on ovaries of either set; but the response from ovaries of frogs kept in water (jar A) was nearly three times as great (39 to 15 eggs) as that from ovaries of frogs kept in moist air. Successive experiments showed that the response from jar B ovaries became progressively weaker with time.

When the combination of jar A pituitaries plus ovaries is compared with that of jar B pituitaries plus ovaries it is seen that the jar A combination is about three times better than that of jar B. This difference, as could be inferred from the above paragraph, is due nearly entirely to the decreased response of the jar B ovaries and not to the decreased potency of the jar B pituitaries.

3. Effect of pH on ovulation.

Measurements of the pH of the pituitary solutions at the end of each experiment gave values averaging around 5.5. Preliminary experiments with buffer solutions showed that good ovulation can be obtained in buffer solutions with pH values around 5.5.

Summary.—1. Excised ovaries from normal female frogs (*Rana pipiens*) will ovulate when placed in Ringer's solution containing pituitaries from *Rana pipiens* frogs. 2. Under the experimental conditions employed it was found that:

a. No ovulation occurred in suspensions of zero, 0.001, 0.002, and 0.005 pituitaries per ml. b. Maximum ovulation occurred at about 0.08 pituitary per ml. c. Little or no ovulation occurred in suspensions of one or more pituitaries per ml. d. In 0.08 pituitary per ml. suspensions good ovulation occurred at pH values around 5.5.

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