INDUCED OVULATION IN *RANA PIPIENS*

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An Abstract

The effect of the anterior pituitary upon the ovulation process in the frog *(Rana pipiens)* was first studied by Wolf (1929). Daily transplants of frog pituitaries into the lateral lymph sac induced ovulation after a variable number of days. Houssay, Giusti, and Lascano-Gonzalez in the same year showed that implanted pituitaries stimulate sexual activity in toads. Rugh (1934, 1935, 1937, 1939) has made studies on the frog which include induced ovulation, quantitative pituitary-ovulation relationships, induced sex reactions, and ovulation times. He states that frogs and toads should be freshly caught while hibernating and used within two weeks. Shapiro and Zwarenstein (1934) observed that toads which were kept in the laboratory longer than three to four weeks appeared to become desensitized to the ovulation producing substance of pregnancy urine. Since our experiences indicated that frogs become less responsive to anterior pituitary injections the longer they are kept in the laboratory a series of experiments were undertaken to test this point in a quantitative manner. To do so, a more accurate test method for the amount of ovulation has been worked out as described below.

The procedure used to induce ovulation is in general the same as that used by Rugh. The method of obtaining counts of the number of eggs ovulated was modified as follows. Instead of weighing the eggs in the body cavity and uteri and the post-ovulation ovaries to obtain percentage ovulation, the ovaries were excised from the frog before ovulation and placed in amphibian Ringer's solution. Counts were made periodically of the number of eggs ovulated in Ringer's and the rate and total ovulation determined.

Mature frogs were kept in a cold-room whose temperature ranged from 12.5 to 17° C. or in a refrigerator at 3 to 6° C. Host females were removed from the cold-room or refrigerator and weighed. The pituitaries for injection were removed from either males or females and placed in distilled water. The standard dose for each injection was four female or eight male pituitaries in one cubic centimeter of distilled water solution. In some cases brain tissue was used for the control injections. Otherwise no injection was made into the controls. A suspension of the glands was obtained by forcing the pituitaries in and out of an hypodermic syringe, and injection was made through a No. 20 needle into the right side of the body cavity of the host. About 15 minutes after injection the host was placed in a bell jar in ¼ inch of chlorine-free tap water and kept in a dark room at about 25° C. Several minutes or hours after injection, depending upon the experiment, the female was single-pithed down the spinal cord and the ventral surface of the body cavity opened. The right and left ovaries were removed by first tying them off at the end of a long thread and dissecting them out at the mesovarium. The ovaries were then suspended just beneath the surface in the Ringer's solution by this thread. At intervals the number of eggs were counted until ovulation ceased. The time of injection was used as the zero point for all subsequent time intervals.

In one series of experiments a group of frogs (I) arrived and were placed in the cold-room at 12.5°C. On subsequent days the degree of ovulation in response to a standard dose of pituitaries was tested. Similarly, for a second group of frogs (II). A third group of frogs (III) were separated upon arrival, some placed in the cold-room and others in a refrigerator at 4°C. The results are shown in table I.

All of these series of experiments indicate that there was a gradual decrease in the amount of ovulation with time when the frogs were kept in the cold-room at 12 to 14°C., but at 4°C. there

TABLE I.—EFFECT OF TEMPERATURE AND TIME UPON OVULATION

	Time since Arrival of frogs in days	Final Number of eggs per ovary			Temperature of cold-room in °C.	Final Number of eggs per ovary			Temperature of refrigerator in °C.
		Right	Left	Aver.		Right	Left	Aver.	
I	3	150	---	150	12.5				
	7	188	46	117	12.5				
	11	0	---	0	12.5				
II	5	742	580	661	13.0				
	13	{347 / 177}	{--- / 168}	230	13.0				
III	6	367	338	353	14.0	298	684	491	4
	11	261	175	218	14.0	86	287	187	4
	15	0	52	26	14.0	411	468	440	4
	25	43	77	60	14.0	338	246	292	4
	28	70	63	67	14.0	476	440	458	4

was as much ovulation on the twenty-eighth day as there was on the sixth day.

In another experiment a group of frogs were kept in a refrigerator at 2°C., and the amount of ovulation was determined from ovaries which were removed from frogs at increasing intervals of time and placed in Ringer's solution. Each frog received a dose of 3 ⅓ female and 3 ⅓ male pituitaries.

TABLE II.—RELATION OF TIME OVARY IS IN FROG TO AMOUNT OF OVULATION IN RINGER'S SOLUTION

Time ovary remained in frog after injection *Minutes*	Average total eggs produced per ovary
22	188
35	461
63	367
121	947
260	1603

These results show nearly a direct proportionality between the length of time the ovary was in the body cavity after injection and the number of eggs ovulated by the ovary after it was placed in the Ringer's solution.

SUMMARY AND CONCLUSIONS

A new method for a quantitative determination of the number of eggs released at any time by excised ovaries is presented.

With this method, the following information was obtained: 1. When frogs which were in hibernation before shipment are kept at 12° C. after arrival in the laboratory, the amount of ovulation obtainable decreases after the first few days. 2. The amount of ovulation obtainable from frogs kept at 4° C. remains relatively constant even after 28 days. 3. Ovaries receive enough hormone to cause some ovulation even though removed from frog within 22 minutes after the equivalent of five female pituitaries was injected into the body cavity. 4. There is nearly a direct proportion between the length of time the ovary is in the body carity after injection and the number of eggs ovulated by the ovary after it is placed in Ringer's solution.

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