INDUCED OVULATION IN *RANA PIPIENS* II.

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The relation of pituitary injection to ovulation in amphibia has been investigated for several years. Rugh (1937) showed that there is a quantitative relationship between the anterior pituitary hormone and the number of eggs ovulated. He expressed the percentage ovulation in terms of the ratio of the weight of eggs found in body cavity and uteri to the weight of the eggs remaining in the ovary plus the weight of the eggs released. The writers (1940) were able to determine directly the number of eggs ovulated by removing the ovary to frog Ringer's solution before ovulation commenced. When the ovaries were removed as early as 22 minutes after injection with a standard dose of pituitary hormone, some ovulation occurred in the Ringer's solution. As the time between injection of the frog and removal of the ovaries was increased the number of eggs subsequently released also increased. These experiments suggested that if the time during which the ovaries remained in the body were held constant and if the amount of hormone injected were varied, a quantitative relation between the hormone and ovulation would be obtained. That this quantitative relationship exists is shown by the following experiments.

Method.—Frogs received during the winter from Vermont and in the spring from Wisconsin were soaked in cold water and then placed in a refrigerator at 5°C. until used. Host females were selected and weighed in order to obtain some index of their ability to ovulate, since Rugh has found a correlation between body size and potency of the female. Pituitary glands for injection were removed from females, placed in distilled water, and broken up into a fine suspension. The various concentrations of the hormone for each experiment were prepared from such a pituitary suspension. Hypodermic injection was made into the mid-coelomic cavity with a No. 20 needle. After injection the host females were placed in a bell jar in ¼ inch of chlorine-free tap water and kept in the darkroom at 24°C. Six hours after injection the host females were single-pithed down the spinal cord and the ventral surface of the body cavity opened. The ovaries were removed and suspended below the surface of frog Ringer's solution. Ovulation occurred in this solution and by periodic counts until ovulation ceased the rate and total amount of ovulation was determined.

Experiments.—On December 22, 1940 a volume of the pituitary hormone suspension was prepared with a concentration of ten pituitaries per ml. In different volumes, doses of 0.3, 0.9, 1.8, 3.8 pituitaries per female were injected. The maximum amount of ovulation occurred with 0.9 pituitary. An increase of the volume by four times (3.8 pituitaries) did not induce a greater amount of ovulation. (See fig. 1 open circles.)

In subsequent experiments the volume of the injection was kept at one ml and the concentration varied from 0.1 to 8.0 pituitaries per ml of suspension. The total amount of ovulation induced by these concentrations is shown in fig. 1.

On February 2, we obtained a typical ovulation curve with pituitary gland equivalent doses of 0.2, 0.4, 0.6, 0.8, 1.0, and 3.5 pituitaries per ml. (See fig. 1 solid circles.) In the concentration range between 0.4 and 0.8 pituitaries per ml the points fell along a straight line indicating that a direct proportionality existed between the concentration of the hormone and the total number of eggs ovulated. Such a proportionality suggests that the method followed in these experiments may be of use as a biological assay for the amount of pituitary ovulation hormone in various tissues and solutions. On March 22 this relationship was confirmed within this same concentration range. (See fig. 1 inverted triangles.) That the curve had a different slope was probably due to the variability between different shipments of frogs. Ovulation reached a maximum at about 1.0 pituitary. This was shown to hold in the previous experiment and was also borne

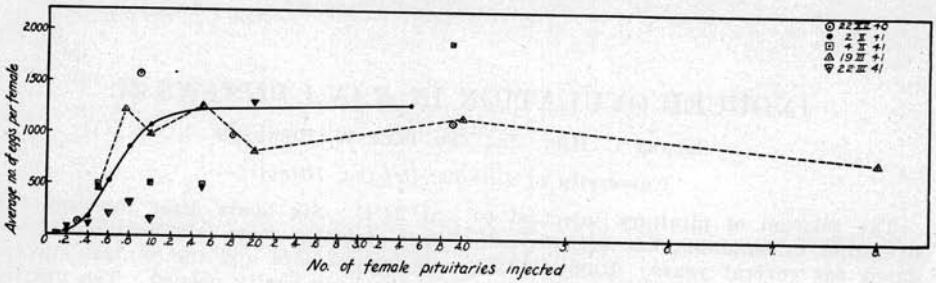

Fig. 1.

out in later ones. It is significant that further increases in the dose even to eight pituitaries induced no greater ovulation. In the lower concentrations, i.e., from 0.1 to 0.4 pituitaries per ml, the points from five experiments all fell close to the typical curve indicating that the response is reproducible in this range.

During March and April frogs showed increased variability in their response to hormone stimulation. Ovulation at this time did not follow closely the curve which was found to be typical in February, the month considered by Rugh best for induced ovulation. Variation of response is caused at least in part by the following factors, more or less difficult to control: The maturity and size of the host female and of the ovary itself (Rugh found larger ovaries more susceptible to hormone stimulation); the amount of available moisture; the storage temperature; the approach of the normal breeding season at which time frogs are less responsive to artificial hormone stimulation. We attempted to control the second and third factors, i.e., the amount of moisture and temperature, by periodically soaking the frogs and by maintaining them at 5°C.

Conclusions.—Using the method of ovulation in Ringer's solution, the effect of pituitary concentrations on ovulation were studied. The results were as follows:

1. In the lower concentrations from 0.1 to 0.4 pituitaries per ml the ovulation response was consistent and increased rapidly with concentration.
2. In the concentration range from 0.4 to 0.8 pituitaries per ml, the total ovulation was directly proportional to the concentration of the hormone. This proportionality suggests that, within this concentration range, a direct biological assay of the pituitary hormone can be made.
3. The maximum amount of ovulation occurred at about 1.0 pituitaries per ml concentration of hormone. Increased concentrations did not induce significantly greater ovulation.

REFERENCES

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