

Summary. Elevated body temperature decreased the level of ascorbic acid in the blood, and also the excretion in the urine. This decreased excretion in the urine as found in this study and as reported for infectious cases with fever would seem to be due to the increased temperature and not to the invading organism.

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Relation of the Intact Pituitary Gland to Artificially Induced Ovulation.

ROBERTS RUGH.*

From Columbia University and Hunter College.

Among the Amphibia the anterior lobe of the pituitary gland has been shown to stimulate ovulation,¹⁻⁵ or amplexus on the part of the male⁶⁻¹¹ and sperm release has also been demonstrated.^{12, 13} Gonadectomy caused morphological changes within the pituitary and hypophysectomy caused gonad atrophy.

It has further been shown¹⁴ that toads will ovulate in response to the pituitary hormone even after hypophysectomy, or the removal of various major organs such as the adrenals, thyroids, kidneys, liver, alimentary tract or parts of the brain. The anterior pituitary hormone seems to act directly on the gonad without the intermedia-

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¹ Houssay, B. A., Giusti, L., and Lascano-Gonzalez, J. M., *Rev. de la Soc. Argentina*, 1929, **5**, 397.

² Wolf, O. M., *Anat. Record*, 1929, **44**, 206; *PROC. SOC. EXP. BIOL. AND MED.*, 1929, **26**, 692.

³ Adams, A. E., *Proc. Second Int. Cong. Sex Research*, 1930, 190.

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

⁵ Shapiro, H. A., *Nature*, 1935, **135**, 510.

⁶ Houssay, B. A., and Giusti, L., *Compt. rend. Soc. de Biol.*, 1930, **104**, 1030.

⁷ Bardeen, H. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **29**, 846.

⁸ Rugh, R., *Biol. Bull.*, 1935, **68**, 74; *J. Exp. Zool.*, 1935, **71**, 163.

⁹ Shapiro, H. A., and Zwarenstein, H., *So. Africa Med. J.*, March, 1935.

¹⁰ Shapiro, H. A., *J. Exp. Biol.*, 1936, **13**, 48.

¹¹ Osima, M., *Sc. Rep. Tohoku Imp. Univ.*, 1937, **12**, 195.

¹² Houssay, B. A., and Lascano-Gonzalez, J. M., *Compt. rend. Soc. de Biol.*, 1929, **102**, 866; *Rev. de la Soc. Argentina*, 1929, **5**, 77.

¹³ Rugh, R., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **37**, 417.

¹⁴ Novelli, A., *Rev. de la Soc. Argentina*, 1932, **8**, 454.

tion of any other organ through either the circulatory or the sympathetic systems. At least 7 variables have been found in this pituitary-ovulation relation¹⁵ and it has long been known that ovarian response is not an all-or-none phenomenon. It was the purpose of this investigation to learn the relation of the intact pituitary to hormonal-induced ovulation in the frog, *Rana pipiens*.

Mature hibernating frogs just received from Vermont were used in the experiments reported here. The anterior pituitary gland was removed, with the frog under ether anesthesia, by approaching it through the roof of the mouth and the parasphenoidal bone. The accompanying diagram shows the roof of the frog's mouth, with oral skin deflected to expose the parasphenoidal and the exoccipital bones and the first vertebra. The anterior pituitary gland is shown as it might be seen, in its normal position, through the parasphenoidal bone. This gland is exposed by 2 incisions, made at about a 30° angle, with sharp point of the scalpel inserted just posterior to the position of the gland. The triangular piece of bone is deflected forward and the anterior lobe of the pituitary gland is exposed and can be sucked up into a narrow-mouthed pipette. In this way the anterior lobe of the pituitary gland can be easily removed without disturbing other parts of the gland, and usually this lobe remains in a single piece. There is a minimum of bleeding if only the anterior lobe is exposed and removed. Care must be exercised, however, to avoid damage to the vena orbitalis posterior or to either of the palatine branches from the carotid artery. These vessels cross the transverse arm of the parasphenoidal bone. Following extirpation of the gland, the triangular flap of bone is replaced and the oral skin is sewed together, with silk thread, to facilitate healing. The controls were operated upon in the same manner, and the pituitary was exposed, but no tissue was removed.

The experiment was divided into 4 graded steps, as follows:

- a. The female frog's anterior pituitary was broken up while in position, by means of a sharp needle. No injection.
- b. The anterior pituitary was removed from the head and immediately injected into the abdomen.
- c. The anterior pituitary was removed and kept in a modification of amphibian Ringer's solution¹⁶ in the refrigerator for a period of 12 hours while the frog recovered completely from anesthetic and operative shock. Then this single pituitary was injected into the abdomen of the frog from which it had been removed.

¹⁵ Rugh, R., *Physiol. Zool.*, 1937, **10**, 84.

¹⁶ Holtfreter, J., *Arch. f. Ent. Mech. der Org.*, 1931, **124**, 404.

d. Procedure same as "c" but the dose of pituitary hormone was increased by using in addition glands excised from other females.

In all cases where anterior pituitary hormone was injected, the procedure was the same as that recently described.¹⁷ The doses recorded are in terms of whole female glands and ovulation was estimated in terms of percentage of the volume of egg mass within the uterus as compared with the total volume of egg mass determined by adding the volume of egg mass remaining within the ovary. The vitelline membrane on the uterine egg partially balanced the ovarian tissue surrounding the ovarian egg, at least to within the

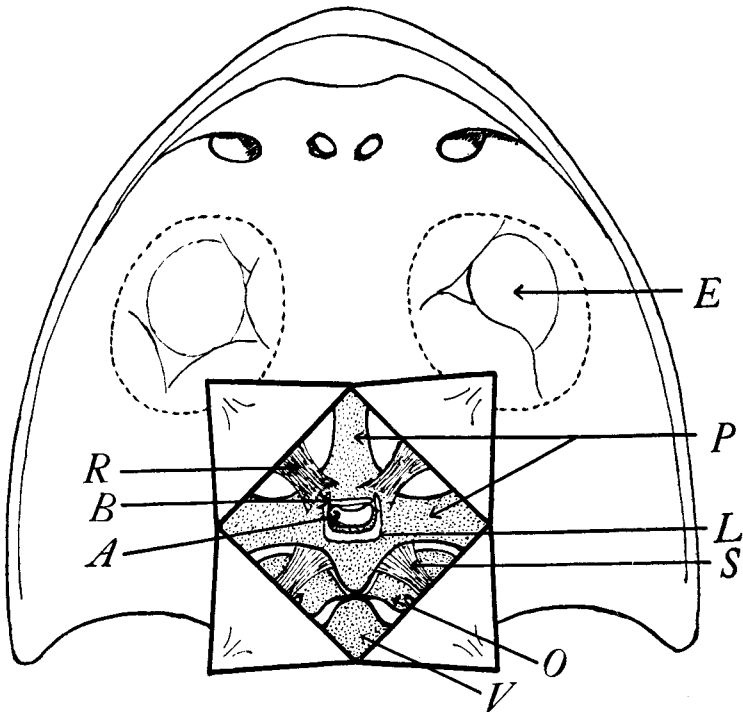


FIG. 1.

Diagram showing position of the pituitary gland of the frog as it might be seen through the parasphenoidal bone, lying just beneath the brain.

A—Anterior lobe of the pituitary gland. Note its more posterior position.

B—Pars intermedia and pars nervosa.

E—Eye ball as seen through oral skin.

L—Endolymphatic tissue adherent to the pituitary.

O—Exoccipital bone.

P—Parasphenoidal bone.

R—Retractor bulbi muscle. The attachment of this muscle to the parasphenoidal bone must be partially removed.

S—Levator anguli scapuli muscle.

V—First vertebra.

limits of observational error, so that this minor factor was not considered. Data were secured 48 hours after hormone injection, a lapse of time found to be sufficient for the completion of ovulation as far as it will go, at laboratory temperatures of approximately 23-25°.

In 4 animals the anterior pituitary gland was broken up in position with a needle, without removal, after exposure as described above. Since this invariably entailed some bleeding it was thought that by this means the hormone might be made immediately available in the circulation. In none of these cases was any ovulation induced. It would hardly be justified, from these observations alone, to say that the host's pituitary would be inadequate to induce ovulation if liberated (as it must be in March) into the blood stream. This operative procedure gave negative results, although the frogs survived for many days.

In 7 animals the gland was removed and injected immediately (even before the frog recovered from the anesthesia) into the abdomen. The double operation (excision and injection) seemed to be too drastic for the frogs. Five died and 2 survived for 48 hours, but were definitely in very poor condition. No ovulation was achieved.

In 5 frogs the anterior pituitary gland was removed and placed in Holtfreter's solution¹⁶ in the refrigerator for a period of 12 hours while the frog made a complete recovery from the anesthetic and the operation. Then this same gland was injected into the frog, inducing some ovulation in every case. The degree to which ovaries were emptied in different frogs varied somewhat. In one case almost complete ovulation was achieved, but the average corresponded well with the tabulated results below from another experimental series.

Since it was found that injection of the anterior pituitary hormone some time after hypophysectomy elicited ovulation, it was necessary to determine the quantitative difference, if any, between the anterior pituitary hormone and ovulation in normal and hypophysectomized frogs. Quantitative studies of this nature have been reported for

TABLE I.

Dose No. of female pituitaries	Controls—Avg % ovulation	Experimentals—Avg % ovulation
1	0-0 0	30-80-50-50 52
2	20-0 10	80-80-95-90 86
3	30-50 40	100-95-100-100 98
4	60-80 70	100-100-95-100 98
8	100-95 98	95-100-100-100 98

*Rana pipiens*¹⁵ but controls were run parallel with hypophysectomized frogs. The controls were operated upon but without gland removal, and received anterior pituitary doses comparable to the doses given to the experimental animals. The results are shown in Table I.

In a subsequent paper the effect of pituitary-induced ovulation on the host's pituitary will be described. Thus far it has been assumed that the host's anterior pituitary gland plays, if anything, a passive rôle in ovulation induced during the hibernation period. It has even been suggested that the hormonal content of the anterior pituitary gland of the hibernating frog may be deficient in respect to the sex stimulating factor. This can hardly be the case since glands from hibernating frogs can be used to induce ovulation as early as the latter part of August in *Rana pipiens*. Also, as indicated above, the host's gland alone is sufficiently potent to induce 50% or more ovulation during hibernation. Recently, hibernating males have been seen to attempt amplexus shortly after being transferred from hibernation temperatures to a level of 11°-14°C. The frog's gland is adequate to cause complete ovulation in March, and it is quite possible that with greater care in the removal, storage, and injection of the one gland that complete ovulation may be achieved during hibernation. It must be admitted, however, that as breeding approaches there may be significant physiological changes both within the pituitary and within the frog as a whole.^{15, 18}

While this pituitary-gonad relation requires further investigation, there appear 2 possible interpretations of the above data. First, the recipient's intact pituitary gland may in some way inhibit the action of added hormone material and this inhibition may be experimentally overcome only by an increase of 300-400% in dosage. Second, there is the hypothetical conception of an inter-relationship between the anterior pituitary gland and the gonads which, when interrupted by the extirpation of one part of the circuit, allows freer invasion of that circuit.

Conclusion. The normal hibernating frog may be induced to ovulate 50% or more of its eggs by the injection of 3 to 4 anterior pituitary glands from female frogs, but if the frog is first hypophysectomized and then allowed to recover completely from the operation, its own gland (or one from another female) will elicit the same degree of ovulation.

¹⁸ Zahl, P., *Z. f. Mikrosk. Anat. Forsch.*, 1937, **42**, 303.