EFFECT OF GONADOTROPIC HORMONES ON HYPOPHYSECTOMIZED (ANTERIOR LOBE) MALE *RANA PIPIENS*

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The reaction of normal male and female amphibia to gonadotropic hormones and to other reagents has received considerable attention within recent years. This study has undoubtedly been stimulated by the importance of the role these animals assumed in pregnancy diagnosis. The successful use of *Rana pipiens* in particular, as a test animal for pregnancy diagnosis has been demonstrated by Wiltberger and Miller (1948). The procedure has as its basis the positive reaction of normal male frogs to injections of gonadotropic hormones, demonstrated by the release of mature spermatozoa in the urine of the frog.

The test for pregnancy just described does not require an understanding of the specific action of the gonadotropins (contained in pregnancy urine) that are introduced into the body of the experimental animal. Do they merely stimulate the pituitary to produce gonadotropic hormones, or act directly upon the gonadal tissue resulting in the release of sperm? There is general agreement, Chu (1946), Simpson (1944), and Oordt (1951), as to the ability of gonadotropins, as well as other specific reagents, to maintain the testicular tissue of hypophysectomized animals, even encouraging spermatogenesis in these animals. However, in reports concerning amphibia, there are no data presented to deny or confirm the ability of the pregnancy urine gonadotropins to effect spermatozoa discharge in hypophysectomized frogs.

To determine more specifically the role played by mammalian gonadotropic hormones in pregnancy tests, experimental male *Rana pipiens* from which the anterior lobe of the hypophysis had been removed, were subjected to the pregnancy test procedure.

METHODS AND MATERIALS

Mature male specimens of *Rana pipiens* were obtained from a commercial source and immediately placed in a refrigerator, simulating hibernation conditions. A total of 180 experimental animals were divided into four categories which were designated as groups A, B, C, and D (table 1).

Group A—A complete control group consisting of 90 frogs which were subdivided into two groups (A' and A'') for the convenience of data tabulation. No surgery was performed on this group.

Group B—A partial control group consisting of 30 frogs. No surgery was performed but injections of distilled water, A.P.L. (anterior pituitary-like substance), and P.U. (a chorionic gonadotropin found in the urine of pregnant women) were administered to this group.

Group C—A partial control group consisting of 20 frogs. Incomplete surgery (anterior lobe of the pituitary left intact) was performed on each member of this group, some of which received injections of distilled water, the others receiving no injections.

Group D—An experimental group consisting of 40 frogs. Complete surgery (anterior lobe of the pituitary removed) was performed on each member of this group, some of which received injections, while others received no injections.

All injected dosages were 4 cc. per frog and consisted either of distilled water or A.P.L., or extracted P.U. The injections were administered subcutaneously by syringe into the lateral lymph sacs of the frog, using a number 23 gauge

needle. Prior to its use, the A.P.L. was diluted with distilled water until a concentration of 25 I.U. of gonadotropins to 1 cc. of distilled water was achieved. Samples of human urine were collected from one individual over a five day period (61st to 65th day of pregnancy). These samples were mixed and gonadotropic hormones extracted from them by the kaolin method.

The frogs to be treated surgically were anesthetized with ether. In this condition the animal was secured to a raised portion of an operating board by a straight pin through the tip of the upper jaw, and retractors applied deep in the mouth over the tongue and on each side of the angle of the jaws (fig. 1). Using a dental drill, a hole was bored through the mid-point of the cross formed by the parasphenoidal and transverse bone of the roof of the mouth. The dura mater in this region was carefully removed with a number 12 scalpel blade, exposing the anterior lobe of the pituitary gland (fig. 2). Preparation of the animal for experimental procedure was completed with the sewing of the flaps of the incised oral skin.

Urine samples were obtained from the experimental animal during the 2 hour post-treatment period by applying moderate finger-tip pressure on the sides of the specimen. These samples were placed on an agglutination slide and examined under a compound microscope for the presence of sperm. None of the frogs, experimental or control, was used a second time—regardless of their reaction.

DISCUSSION

While the absolute necessity of the presence of the pituitary gland to maintain life has not been entirely determined, workers in the field of endocrinology have clearly demonstrated that the removal of the gland results in extensive abnormalities. "Spermatogenesis is entirely dependent on a secretion of the anterior pituitary gland. . . . In the absence of the anterior pituitary gland follicular growth is limited or absent, and the ovaries atrophy" (Winton and Bayliss, 1949).

Sexual stimulation, in the frog, is associated with the secretion of certain hormones by the pituitary gland, one of which, the interstitial cell stimulating hormone (ICSH), is held to be directly responsible for the actual release of mature spermatozoa from the germinal epithelium of the testis. It has been established, (Wiltberger and Miller, 1948), that in the case of *Rana pipiens*, another hormone, extracted from the urine of pregnant women, also produces a gonadotropic effect and constitutes the basis for the male frog human pregnancy test.

We recognize, therefore, on one hand a hormone (ICSH) as being produced by the pituitary and acting directly upon the testis of a normal male amphibian, while on the other hand there is evidence of an identical response attributed to still another hormone (P.U.) introduced into the body of the frog. The question then poses itself as to whether this identical reaction is due to the direct action of P.U. on gonadal tissue, or an *indirect* one, wherein this hormone functions merely as an agent which stimulates the pituitary gland to produce ICSH, eventually causing the release of spermatozoa.

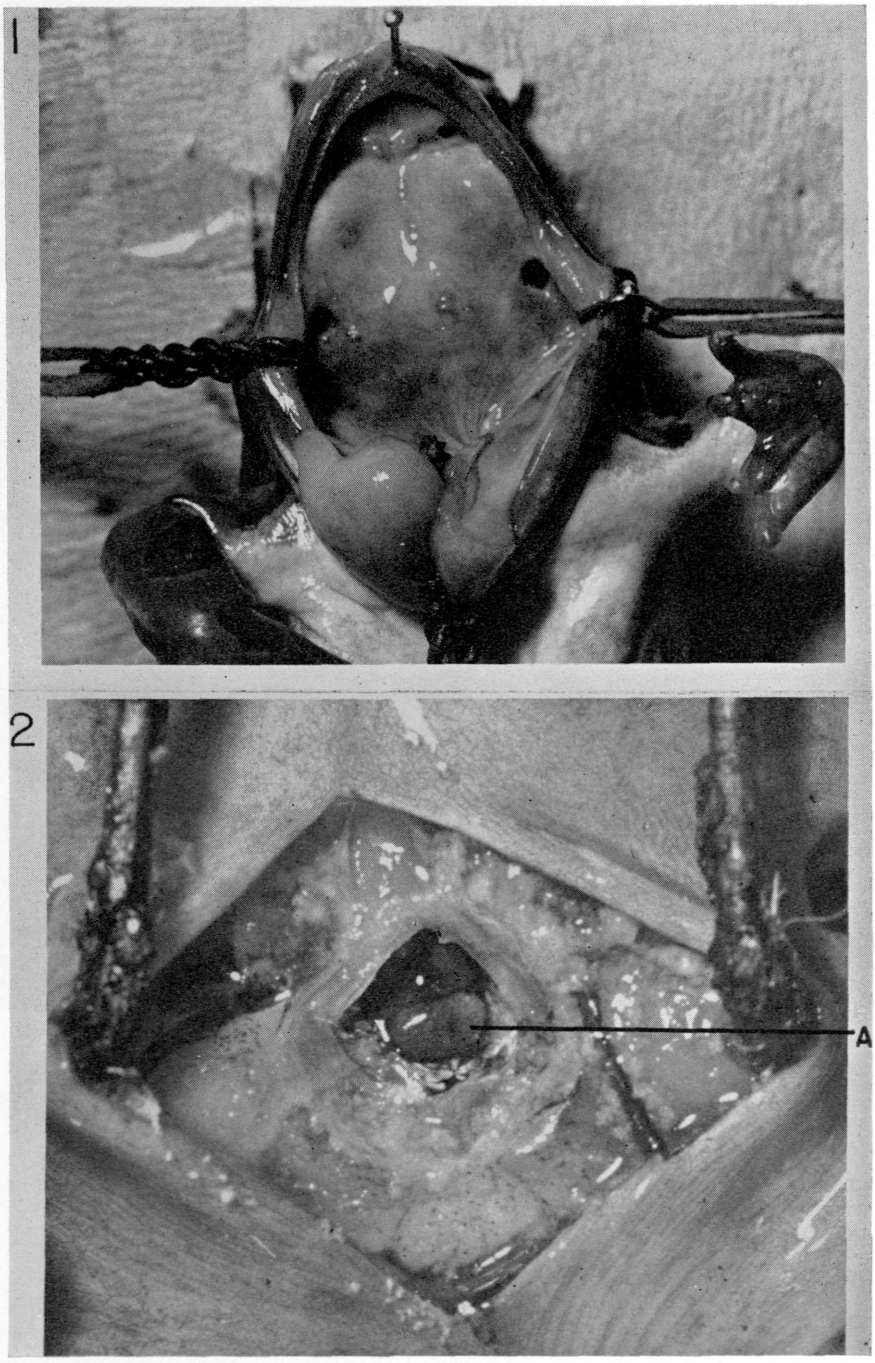
The results included in Table 1 may be summarized as follows:

Normal male *Rana pipiens* recently removed from hibernation,

1) respond negatively (groups A', A'' and B) when (a) untreated, or (b) injected with 4 cc. of distilled water;

EXPLANATION OF FIGURES IN PLATE

1. Position of the frog on the operating board prior to the removal of the anterior lobe of the hypophysis. Note the straight pin through the tip of the upper jaw, and retractors applied deep in the mouth over the tongue and on each side at the angle of the jaws.
2. Appearance of operation site following the removal of the thin section of bone and/or dura mater of the brain which covers the area occupied by the anterior lobe of the hypophysis. A, anterior lobe of the hypophysis.


1
2
A

2) respond positively (groups B and A") in tests for the presence of spermatozoa in the urine when (a) injected with A.P.L., or (b) injected with P.U.;
3) and when treated surgically short of pituitary removal (group C) respond negatively in tests for the presence of spermatozoa in the urine when (a) uninjected, or (b) injected with 4 cc. of distilled water;
4) and undergoing anterior lobe hypophysectomy (group D) (a) respond negatively in tests for the presence of spermatozoa in the urine when (1) uninjected, or (2) injected with 4 cc. of distilled water; (b) respond positively for the presence of spermatozoa in the urine (as long as 5 days after surgery) when (1) injected with 4 cc. of A.P.L., or (2) injected with 4 cc. of P.U.

TABLE 1

Reactions of male Rana pipiens to all conditions of the experiment

Groups	No. of Frogs	Reading	Dist. H_2O Reaction		A. P. L. Reaction		P. U. Reaction		Uninjected	
			+	−	+	−	+	−	+	−
A'	30	2 hrs.							0	30
B	30	2 hrs.	0	10	10	0	10	0		
A"	60	2 hrs.	0	20	10	0	10	0	0	20
C	20	2 hrs.	1	9					0	10
D	40	2 hrs.	0	10	10	0	10	0	0	10
		5 days					10	0		

In addition to gonadotropins, sperm release in the male *Rana pipiens* has been observed following sufficient injections of distilled water (Giltz and Miller, 1950). However, the negative reaction of groups B, C, and D to injections of 4 cc. of distilled water indicates that the positive results obtained from both normal and hypophysectomized animals following injections of 4 cc. of the gonadotropic hormones are not expressions of the animals to the quantity but rather to the quality of the liquid introduced.

Although no report of sperm emission, resulting from surgical treatment, was found in the literature, the possible association of this factor with the occurrence of false positive results could not be overlooked. Observation of the effects of partial and complete surgery, alone, and followed by injections of 4 cc. of liquid, was accomplished by subjecting smaller units of group C and D to this sequence of treatments.

The negative reactions displayed by these groups eliminate the quantity of liquid injected in combination with the effects of surgical treatment as being factors which might yield false positive results.

The reactions of the experimental specimens of group D and of the partial control group B to injections of 4 cc. of A.P.L. and P.U. are all positive. This would seem to indicate that the presence of the anterior lobe of the hypophysis is not essential in the phenomenon of sperm emission by male *Rana pipiens* following the injection of the above mentioned hormones into the body of the frog. It must be kept in mind, however, that at the time of hypophysectomy, and for a period of time thereafter, a quantity of pituitary-produced hormones may still exist in the body fluids of the experimental animals. Since all animals were tested after only a brief post-operative interval, the question might be raised

as to whether the positive reaction demonstrated by group D is an expression of the specific effect of the injected gonadotropins, or merely the result of a chain reaction in which the introduced hormones function solely in the activation of residual pituitary-produced hormones. An attempt to resolve this question was made by maintaining a portion of the animals in group D at room temperature for a post-operative period of five days. At the end of this period the animals were again treated with injections of gonadotropic hormones. The responses of the animals in this category are still positive and it can be reported that there was no apparent decrease in the intensity of the reaction after the period of five days. An equal number of normal frogs were maintained in a similar manner to determine the effects, if any, of exposure to room temperatures. There was no instance of spermatozoa emission in any of these control animals.

SUMMARY

The results of the experimentation with gonadotropins upon anterior lobe-hypophysectomized male *Rana pipiens* may be summarized briefly as follows:

1. The phenomenon of spermatozoa release, initiated by injections of mammalian gonadotropic hormones, can be accomplished in the absence of the anterior lobe of the hypophysis.
2. The specific role played by mammalian gonadotropic hormones in the pregnancy tests is believed to be a direct one upon the gonadal tissue, resulting in the emission of mature spermatozoa.

REFERENCES

Chu, J. P. 1945. Gonad stimulation by androgens in hypophysectomized pigeons. Jour. of Endocrin., 4: 431–438.

Giltz, M. L., and **D. F. Miller.** 1950. Ways of improving the male frog test for pregnancy. Ohio Jour. Sci., 50: 205–209.

Oordt, G. J. 1951. Spermatogenesis in normal and hypophysectomized frogs (*Rana temporaria*) following gonadotropin administration. Acta Endocrin., 7: 257–269.

Simpson, M. E. 1944. Sensitivity of the reproductive system of hypophysectomized 40 day male rats to gonadotropic substances. Endocrin., 35: 96.

Wiltberger, P. B., and **D. F. Miller.** 1948. The male frog, *Rana pipiens*, as a new test animal for early pregnancy. Science, 107: 198.

Winton, F. R., and **L. E. Bayliss.** 1949. Human Physiology. The Blakiston Co., Phila., 3rd Ed., 279–281.