PHYSIOLOGICAL PROCESSES ASSOCIATED WITH SPERM EMISSION IN THE LEOPARD FROG, *RANA PIPIENS*[1]

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The male frog test for pregnancy, announced by Wiltberger and Miller (1948), and Robbins and Parker (1948), has caused some investigators to try to determine the nature of the physiological processes involved in the emission of the spermatozoa. Among these were Robbins and Parker (1949) who reported the emission of spermatozoa following injections of adrenergic substances; Giltz and Miller (1950) who noted the emission of spermatozoa following the injection of distilled water, pond water or tap water in amounts exceeding 20 percent of the body weight of the frog; Biesinger and Miller (1952) who studied histological sections of the testes before and after injections of pregnancy urines; McCourt (1953) who sectioned the testes longitudinally and found that there is an anterior-posterior gradient of the mature tubules of the testes; and Kissen (1954) who reported the emission of spermatozoa in the urines of hypophysectomized frogs following the injection of either pregnancy urines or anterior pituitary-like hormones.

Notwithstanding these investigations on *Rana pipiens*, and the inferences to be drawn from earlier work on pituitary-gonad relationships of other species of frogs, which was summarized by Houssay (1949), the exact manner in which gonadotropins influence the urogenital system was not yet determined. It is not generally believed that adrenergic agents, water, pituitary substances, and the pregnancy urine hormone would produce the same physiological effects upon all animal tissues, yet the effects of these substances upon the frog are all correlated with the emission of spermatozoa in its urine.

The results of the following investigation help to clarify the part which the above substances play in bringing about the movement of spermatozoa from the testes to the bladder. The first area of investigation was devised to study excised testes, which were observed in order to determine whether there was an emission of spermatozoa from the testes into various fluids in which they were immersed. The second area of investigation involved a study of testes in situ following gonadotropic and non-gonadotropic subcutaneous injections. The third area of investigation involved frogs which had partial or entire brain extirpations preceding subcutaneous injections of either gonadotropic or non-gonadotropic substances.

Materials. The frogs used in these experiments were the leopard frog, *Rana pipiens*, procured from commerical suppliers. They were stored in groups of 25 in small aquaria in a refrigerator at approximately 45° F. Ayerst brand of chorionic gonadotropin ("APL") was used. It contained 100 international units (I.U.) per cc. of solution. It was diluted with distilled water or saline solution according to the number of international units desired for the various experiments. The saline solution used was Ringer's solution for cold blooded animals, prepared according to Lee (1950). The adrenalin used was Parke, Davis and Company, 1: 1000. The human pregnancy urines (H.P.U.) were from samples submitted for pregnancy diagnosis.

The emission of spermatozoa from excised testes. Glass and Rugh (1944), Biesinger and Miller (1952), and McCourt (1953), have made histological studies of testicular tissue which was removed from treated and untreated frogs. In these studies a change in position of spermatozoa from the periphery of the

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tubules, where they are attached to Sertoli cells, to the center of the tubules, where they are usually free from the Sertoli cells, was associated with the injection of gonadotropins. However, the association of this reaction with injections does not preclude the possibility that the injections act on some structure other than the testes. In my study experiments were devised to determine whether gonadotropins have an effect upon the movement of spermatozoa from the Sertoli cells at the periphery to the lumen of the tubules and out of the excised gonads.

Before the testes were excised various solutions were prepared, and one cubic centimeter of each solution was placed in a concavity slide. The solutions were: saline, distilled water, frog urine, frog plasma, human pregnancy urine, human non-pregnancy urine, saline plus various amounts of anterior pituitary-like chorionic gonadotropin, distilled water plus various amounts of anterior pituitary-like chorionic gonadotropin, saline plus various amounts of adrenalin, and distilled water plus various amounts of adrenalin. Each excised gonad was placed in a concavity slide containing one of the solutions. and care was taken to insure their

TABLE 1

The effect on emission and motility of spermatozoa obtained when excised testes were immersed in various solutions

Approx. No. of Trials	Immersion Solution	Effect	
		Emission of Spermatozoa	Motility of Spermatozoa
150	Distilled water	Positive	Positive
10	Tap water	"	"
70	Frog urine	"	"
70	Distilled water plus A.P.L.	"	"
50	Distilled water plus adrenalin	"	"
10	50% saline and 50% distilled water	"	"
150	Saline	Negative	Negative
70	Saline and A.P.L.	"	"
50	Saline plus adrenalin	"	"
35	Pregnancy urine	"	"
25	Fluid found in body cavity of frog	"	"
35	Lymph	"	"
25	Serum	"	"

submergence. Each preparation was examined microscopically for emerging or emerged spermatozoa. When this examination revealed that spermatozoa were streaming constantly or intermittently from the testes, the observation of that testis was recorded as positive. When the spermatozoa in the immersion fluid were motile it was recorded as positive for activity. When spermatozoa did not emerge from a testis the observation of that testis was recorded as negative. At times a few (2 to 50) spermatozoa were present from manipulation when the solutions were first observed. These had to be discounted in the results on emission but a record was made of their motility. Spermatozoa were motile if emission was taking place and immotile if emission was not taking place.

The experiments on emission were carried out during the months from February to November inclusive, at temperatures between 50° and 95° F. Neither season nor temperatures between these degrees appeared to influence the number of testes responding to treatment, nor did they influence the degree or number of spermatozoa which were motile. Investigators using intact frogs have found a seasonal sensitivity to gonadotropins (Sampson 1950). It was also shown that fewer

frogs emitted spermatozoa if the temperature of the room in which they were kept following the injections was above 80° F (Giltz and Miller 1950). Since season and temperature affect the emission of spermatozoa from intact frogs and not from excised testes it must be concluded that seasonal and temperature differences are due to an affect on structures other than the testes.

The experiments summarized in table 1 show that spermatozoa flow out of testes when placed in distilled water, tap water and frog urine. Water is obviously hypotonic to frog blood, and the urine of the frog is normally very dilute (Prosser *et al.*, 1950). Adolph (1927) found that the urine of *Rana pipiens* is strongly hypotonic to other body fluids even when the frog is in a hypotonic medium. Therefore, the fluids which are associated with the emission of spermatozoa are all hypotonic to the blood of the frog.

From the data in table 1, it is also shown that spermatozoa do not flow out of testes placed in saline solution, frog lymph, frog serum, fluids sometimes found in the frog's body cavity, and human pregnancy urines. It also indicates that the addition of anterior pituitary-like hormone and adrenalin to these solutions does not stimulate the emission of spermatozoa. These fluids into which spermatozoa do not flow from the testes are all isotonic or hypertonic to the blood of the frog.

TABLE 2

Effects of various solutions on the size of excised testes

Number Testes	Solution in Which Testes Were Immersed	Effect of Immersion on Sizes of Testes	Spermatozoa Emission
24	Saline	No increase	Negative
12	Saline plus adrenlin	" "	"
24	Saline plus A.P.L.	" "	"
20	Human pregnancy urine	" "	"
12	Frog blood serum	" "	"
18	Frog lymph	" "	"
9	Fluid sometimes found in body cavity	" "	"
14	Distilled water plus adrenlin	Increase	Positive
24	Distilled water plus A.P.L.	"	"
27	Distilled water	"	"
16	Frog urine	"	"

Since spermatozoa do emerge from excised testes immersed in hypotonic solutions and do not emerge in isotonic or hypertonic solutions regardless of the addition of pharmaceutical substances, it is concluded that the hypotonicity of the fluid is the dominating factor in the release of spermatozoa from excised testes.

The volume of excised testes associated with the emission of spermatozoa. During the experiment on the emission of spermatozoa from excised testes it was noticed that the testes which emitted their spermatozoa appeared to increase in size. It was also noted that testes, which at the beginning of the experiment were smaller at one end than the other, became more symmetrical when they were submerged in solutions into which they discharged their spermatozoa.

If there was an actual increase in size it would lend support to the hypothesis that diffusion into testicular tissue was taking place when the testes were subjected to solutions which are hypotonic to body fluids of the frog. Therefore, experiments were devised to determine whether there is an increase in the volume of excised testes which can be correlated with the emission of spermatozoa. These testes were measured at the time they were immersed in the various solutions and again after an interval of an hour in the solutions. The measurements were made by placing the concavity slide containing a testis on a millimeter rule and reading the

length and width from the sides and ends of the testes through a monocular microscope.

The results of the measurements are summarized in table 2. When the testes were placed in saline, saline plus adrenalin, saline plus anterior pituitary-like hormone, human pregnancy urines, frog blood serum, frog lymph, or fluids sometimes found in the body cavity of the frog, there was no increase in the volume of the testes as determined by measurements and no emission of spermatozoa. When the testes were placed in distilled water plus adrenalin, distilled water plus A.P.L., distilled water alone, or frog urine, all testes increased in volume as determined by measurements and emitted spermatozoa.

Since the emission of spermatozoa is correlated with an increased testis volume as well as the presence of hypotonic solution it appears that this strengthens the hypothesis made earlier that the release of spermatozoa is due to the diffusion of a hypotonic solution into testicular tissue. It appears that the diffusion of the hypotonic solution into testicular tissues may be the cause of the increased size. It also indicates that the emission of spermatozoa can be explained without the assumption of smooth muscles in the testes, for if smooth muscles alone were involved there would be a decrease in the size of the testis in at least one dimension.

TABLE 3

Summary of the effects of subcutaneous injections on the size of the testes and the emission of spermatozoa into the urine.

Substance Injected	Testes Measured	Testes Increasing in Size	Testes Not Increasing in Size	Frogs With Sperm in their Urine	Frogs With out Sperm in Urine
2 cc. 100 I.U. A.P.L. in Dist. Water	24	22	2	11	1
2 to 4 cc. of Dist. Water	20	0	20	0	10
10 cc. of Dist. Water	20	20	0	10	0
10 cc. of Saline	20	0	20	0	10
4 cc. of Raw Pregnancy Urine	20	18	2	9	1
4 cc. of non-Pregnancy Urine	20	0	20	0	10

These conclusions suggest that a similar pressure action by hypotonic fluids might be associated with the emission of spermatozoa from intact testes. If this were the case one would expect an increased size of testes in frogs which release their spermatozoa following injections of gonadotropins. In order to determine this the next series of experiments was undertaken.

The relation of the volume of testes in situ to the emission of spermatozoa. Testes in situ were measured preceeding and following subcutaneous injections of (a) 100 I.U. of anterior pituitary-like hormones in 2 cc. of distilled water, (b) 4 cc. of human pregnancy urine, and (c) 10 cc. of distilled water. These testes increased in volume and emitted spermatozoa. The increases averaged 0.4 mm. in length and 0.15 mm. in width or depth. Similar measurements were made on testes in situ of frogs receiving subcutaneous injections of (a) 2 cc. of distilled water, (b) 4 cc. of non-pregnancy urine and (c) 10 cc. of saline. These testes did not increase in volume as determined by measurements nor did they emit spermatozoa. This experiment is summarized in table 3. The increase in volume is therefore

associated with an emission of spermatozoa in intact testes. The increased volume can be explained on the basis of an increased diffusion pressure resulting from the entrance of a hypotonic fluid into the testis. In this experiment the frogs which were injected with substances associated with a release of spermatozoa were the only ones whose testes increased in size. Therefore, these substances, anterior pituitary-like hormones, human pregnancy urines, and water in 10 cc. amounts, are believed to act on the structure of the urogenital system in such a manner that a hypotonic fluid is introduced into the testes.

Observations on the urogenital system of injected frogs. Observations were made on the urogenital system of frogs which had received a subcutaneous injection of 100 I.U. of anterior pituitary-like hormones or 3 cc. of raw human pregnancy urine. When the frogs received these injections, the glomeruli and Bowman's capsules swelled and became visible through the surface of the kidney. This was associated with an increase in testis volume as well as an emission of spermatozoa. However, when similar observations were made on frogs which received an injection

TABLE 4

The effect of neural extirpations on the release of spermatozoa

Part of Brain Extirpated	Approx. No. of Trials	Injection Solution	No. Time Spermatozoa were Recovered in Urine
Entire brain	65	A.P.L. or H.P.U.	0
Midbrain	40	A.P.L. or H.P.U.	0
Optic lobes only	70	A.P.L. or H.P.U.	0
Transection posterior to optic lobes	20	A.P.L. or H.P.U.	0
Transection of spinal cord to posterior to medulla	30	A.P.L. or H.P.U.	0
Forebrain	60	A.P.L. or H.P.U.	60
Cerebellum only	10	A.P.L. or H.P.U.	10
Pituitary only	20	A.P.L. or H.P.U.	20
Transection anterior to optic lobes	45	A.P.L. or H.P.U.	45

of either 2 cc. of water or non-pregnancy urine, there were no swellings in either the glomeruli or the capsules, nor did these testes increase in size or release their spermatozoa.

Richards *et al.* (1927), determined that the addition of adrenalin in certain titers resulted in a swelling of the glomeruli. This was due to the constricting action of adrenalin on the efferent vessel of the glomerulus. Since the leopard frog responds to adrenergic substances, human pregnancy urine and anterior pituitary-like hormone, by releasing spermatozoa and the glomeruli swells when these substances are applied it may be possible that the action of anterior pituitary-like hormones and human pregnancy urines may also cause the constriction of the efferent vessel of the glomerulus increasing the pressure in the urogenital circulation.

Experiments with brain extirpations. The effect of various brain operations on the emission of spermatozoa from the testes to the bladder were studied. When

the olfactory lobes, cerebral hemispheres, pituitary and cerebellum were extirpated there was an emission of spermatozoa following the injection of gonadotropins. However, when the entire mid-brain or the optic lobes alone were extirpated there was no emission of spermatozoa. Therefore, it was concluded that the optic lobes exerted a neutral control on the urogenital system. In the light of the evidence of the stimulating effect of hypotonic solutions on the emission of spermatozoa it appears that the influence of gonadotropins is exerted on the kidney. If this were the case the destruction of the optic lobes may destroy or inhibit the muscular tone of the vascular system of the kidney. The results of this experiment are summarized in table 4.

It is believed that this view is supported by the results of an experiment on the effects of subcutaneous injections of water on frogs with pithed and extirpated optic lobes. Table 5 is a summary of this experiment which indicates that 10 cc. amounts of distilled water stimulates the emission of spermatozoa even though

TABLE 5

The effect of 10 cc. of saline and 10 cc. of water on the emission of spermatozoa in frogs with either optic lobe extirpations or pithed brains.

Part of Brain	Number of Trials	Injected Solution	Number Times Spermatozoa Were Recovered in Urine
Entire brain, pithed	10	10 cc. water	10
Optic lobes only extirpated	5	10 cc. water	5
Entire brain pithed	10	10 cc. saline	0
Optic lobes only extirpated	5	10 cc. saline	0

the neural associations have been destroyed. This would indicate that either water has a greater affect on the muscles of the kidney than anterior pituitary-like substances and human pregnancy urine or that it is available to the testes through dilution of the blood and increase in vascular pressure even though the optic lobes were destroyed.

CONCLUSIONS

From the results of the experimentations on excised testes, intact testes, and injections following brain extirpations the following conclusions appear to be justified:

1. Hypotonic fluids are associated with the release of spermatozoa from the testis.
2. An increase in the volume of the testis is associated with the movement of spermatozoa from the testis to the urine.
3. Gonadotropic substances are not associated directly with the emission of spermatozoa from the testis.
4. The association of an increase in testis size with the emission of spermatozoa seems to indicate the presence of a diffusion pressure within the testis.
5. There is visible evidence that seems to indicate that gonadotropic substances directly affect the vascular system of the kidney when spermatozoa are emitted.
6. In the normal frog the release of spermatozoa, following the injection of gonadotropic substances, is influenced by the optic lobes because the extirpation of the lobes destroys the release mechanism.

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