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Facilitation of Pituitary-Induced Frog Ovulation by Progesterone in Early Fall.* (26333)

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Since the original investigations of Wolf (1) on *Rana pipiens* and Houssay *et al.* (2) on *Bufo arenarum*, induction of ovulation in anurans by homoplastic pituitary injections or implantations has become a common laboratory procedure. Freshly-ovulated eggs for fertilization studies and embryological material may be obtained at any time during the academic year, but minimal effective dosages of pituitary glands vary widely with the season (3). Nearly complete ovulation in March or April may be induced in the average frog 24 hours following injection of 1 or 2 frog anterior pituitary lobes, but 3 to 4 lobes are usually required in January and February. From mid-September often into December, significant ovulation in a single female necessitates slaughter of 6 to 8 other females, only for their pituitary glands; and 48-56 hours must ordinarily elapse following injection before any appreciable response is obtained. Recent success with steroids in inducing ovulation *in vitro* in *Xenopus laevis* (4) and in *Rana pipiens* (5,6) has quite naturally led into a study of possible use of steroid hormones as substitutes for part or all of the large pituitary dosages required for ovulation *in vivo* in the fall.

Methods. Frogs for these studies were obtained from both Vermont and Wisconsin. Suppliers were instructed not to ship animals which had been kept in a warm room. Frogs

were stored upon arrival in large aquaria in a refrigerator (5°C) with a little water, changed frequently. Pituitary glands were dissected from freshly-killed animals, ground in a small mortar, and injected (#27 needle) in a small volume of Ringer's solution into the dorsal lymph sac. Progesterone or other steroid, as aqueous suspension or dissolved in oil, was administered similarly. Following injection, each animal was kept at room temperature in a large finger bowl containing about a half inch of water and covered to prevent escape but loosely enough to admit air. Evidence of ovulation was often indicated by ability to force ova from the ovisacs, but percentages of ovulation were determined by laparotomy.

Results. Very satisfactory responses (Table I) were obtained when frogs were injected with a single pituitary gland in combination with from 0.5 to 5.0 mg progesterone. Ova could be stripped in profusion from recipients within 12-16 hours following injection. The fact that these ova were readily fertilizable and underwent normal development into swimming larvae indicates that ovulation so obtained is entirely normal. At the time of laparotomy (24 hours following injection), the coeloms of these animals were still crowded with freshly-ovulated eggs, signifying that abundant ovulation was still in process.

Small amounts of ovulation were also obtainable by injecting progesterone alone

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TABLE I. Ovulation Induced in *Rana pipiens* in October by Injection of One Triturated Frog Pituitary Gland in Combination with Progesterone.

Progesterone dosage, mg	No. frogs	No. frogs responding	Avg % ovulation*
0	6	0	0
.1	4	1	2
.5	"	4	40
1.0	"	"	60
2.0	"	"	75
5.0	"	"	85

* Determined by laparotomy 24 hr following inj.

TABLE II. Ovulation Induced in October by Injection of Progesterone Alone.

No. progesterone injections	Total progesterone admin., mg	No. frogs	No. frogs responding	Avg % ovulation*
2	1	2	0	0
2	2	2	1	1
1	5	3	3	5
2	10	4	4	10
2	20	2	2	1
2	25	2	0	0
1	25	2	Died	—

* Determined by laparotomy 48 hr after first inj.

(Table II), but this steroid obviously is not an effective substitute for pituitary factors, at least in October. It is interesting that optimal response, even though it was only about 10%, followed a total dosage of 10 mg, and that larger amounts were apparently inhibitory. A single dosage of 25 mg progesterone was lethal within 1 hour. Those animals giving positive responses to progesterone alone had a tendency to force ova from the ovisacs spontaneously, whereupon the jelly coat begins to swell immediately; such eggs are of no use as potential embryological material since they cannot be fertilized. Testosterone in oil, in total dosages of 5 and 10 mg, also induced 5-10% ovulation (4 frogs), but larger amounts seemed to be even more toxic than progesterone and did not induce ovulation.

Discussion. Progesterone's ability to augment the ovulatory effect of pituitary factors is evident in the foregoing data. It is particularly interesting that progesterone markedly reduced both the dosage of pituitary hormone (by a factor of 6 or more) and

time required for maximal response (by a factor of 2 or 3). Although these experiments were performed in October, there is every reason to suppose that similar results could be obtained in September or even August. Since pituitary-induced ovulation in October from frog ovarian lobes *in vitro* was also found to be augmented by progesterone (by a factor of 3), it is assumed that the effects noted *in vivo* are direct and not mediated by the recipient's own pituitary gland. The possibility remains, however, that progesterone could facilitate release of pituitary gonadotropin as has been observed in rabbits(7) and fowl(8). A repeat of the experiments in Table I, substituting testosterone in oil for progesterone, gave positive results (40% ovulation) only at the 5 mg steroid level. Substitution of an aqueous suspension of cortisone[†] for testosterone in another repeat gave only 1-2% ovulation at both 2 and 5 mg steroid dosages. It is notable, though, that a frog injected with one pituitary gland plus 2 macerated frog adrenal glands ovulated completely in 24 hours. Apparently, then, cortical and androgenic steroids in proper dosage may also facilitate pituitary-induced ovulation *in vivo*.

Summary. In October, when *Rana pipiens* ovaries are notoriously insensitive to ovulatory influences, injection of one frog pituitary gland in combination with small dosages of progesterone induced nearly complete ovulation within 24 hours. Progesterone, and to a lesser extent testosterone, also exerted some ovulatory effect when injected alone.

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