

## Effects of Administering Mouse Anterior Pituitary to the Newt and the Frog.\*

A. ELIZABETH ADAMS, ELIZABETH M. DEFORST AND BARBARA GRANGER.

*From the Department of Zoology, Mount Holyoke College, South Hadley, Mass.*

Induction of extra-seasonal ovulation in anurans and urodeles has been secured by injecting extracts of mammalian anterior pituitary<sup>1</sup> or of human pregnancy urine<sup>1</sup> although in some instances no response occurred.<sup>1, 2</sup> Attempts to cause the reaction with implants of fresh mammalian gland (sheep, cattle, dog, guinea pig, rat, rabbit<sup>3</sup>) have given negative results. Further tests of fresh substance were, therefore, made in which mice were donors and 2 amphibians, *Triturus viridescens* and *Rana pipiens*, hosts.

*Triturus viridescens* as hosts. (Experiments performed by E. DeF.) Between October, 1938, and March, 1939, 24 adult female newts received implants of fresh anterior pituitaries of adult male mice in varying amounts and spaced at varying intervals. Five died on the 2nd, 5th or 6th day of the experiment and one at autopsy had an ovary filled with atretic eggs. Of the 18 remaining, 6 ovulated and 5 of these laid eggs (Table I). The first egg-laying occurred at an average of 11.2 days. The average number of eggs ovulated was 10.3 and laid was 4.6 per newt. The average number of APs given was 12.5, a dosage of 12.6 mg of substance.

As controls 16 newts received implants of newt whole pituitaries spaced to correspond with the mouse implants. Four did not ovulate, but 3 of these died within 2 to 5 days after treatment and in the 4th the ovaries were immature. The remaining 12 laid eggs after an average of 6.1 glands (approximately 0.84 mg of substance<sup>4</sup>) and of

\* Aided by a grant from the Rockefeller Foundation.

<sup>1</sup> See Adams, A. E., *Studies in Experimental Zoology*, 1936, Edwards Brothers, Ann Arbor, pp. 65-67, and Rugh, R., *J. Exp. Zool.*, 1935, **71**, 149, Table 3 for bibliography.

<sup>2</sup> Adams, A. E., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28**, 677; Baker, L. C., *J. Tenn. Acad. Sci.*, 1937, **12**, 206; Creaser, C. W., and Gorbman, A., *Copeia*, 1936, p. 91; Gallien, L., *Compt. rend. soc. biol.*, 1937, **124**, 874; Zondek, B., *Hormone des Ovariums und des Hypophysenvorderlappens*, J. Springer, 1935.

<sup>3</sup> Adams, A. E.;<sup>2</sup> Houssay, B. A., Giusti, L., et Lascano-Gonzalez, J. M., *Compt. rend. soc. biol.*, 1929, **102**, 864; Wills, I. A., Riley, G. M., and Stubbs, E. M., *Proc. Soc. Exp. Biol. and Med.*, 1933, **30**, 784; Witschi, E., *Cold Spring Harbor Sympos. Quant. Biol.*, 1937, **5**, 180.

<sup>4</sup> Figure computed from data in paper by Adams, A. E., and Granger, B., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 552.

TABLE I.  
*Triturus viridescens* Given Intramuscular Implants of Adult Male Mouse Anterior Pituitaries or of Male Newt Whole Pituitaries.

No. of newts	Treatment	Anterior pituitaries		Day of autopsy	Eggs			Actual or estimated <sup>1</sup> wt of both ovaries (mg)
		No.	Total wt (mg)		laid	in body cavity or oviducts	in ovaries	
Donor Mouse								
1	1 AP daily	11	12.78	12	0	13	109.0	474.00
1	1 " "	11	11.79	12	0	1	127.0	448.51
1 <sup>2</sup>	1 " "	13	13.26	14	7	2	115.0	359.34
8 <sup>3</sup>	1 " alternate days	15	avg 12.97	{ 31	0	0	avg 278.5	avg 693.65
				{ 34				
1	2 " daily	16	11.60	9	1	1	176.0	575.72
1	2 " alternate days	12	14.14	12	22	6	93.0	453.80
2	2 " "	16	avg 13.03	16	0	0	avg 170.5	avg 566.90
1 <sup>4</sup>	Single dose	14	12.2	10	0	0	104.0	370.80
1 <sup>5</sup>	" "	15	12.8	10	0	0	135.0	461.80
1	" "	24	11.8	13	9	0	151.0	462.52
Donor Newt								
			Whole pituitaries					
1	1 AP daily	11	1.516	13	0	77	35.0	456.96
1	1 " "	11	1.51	15	0	93	33.0	431.54
1 <sup>2</sup>	1 " "	11	1.51	12	2	22	106.0	480.60
1 <sup>2</sup>	1 " "	13	1.78	14	1	17	116.0	398.10
4	1 " alternate days	15	2.06	{ 31	avg 76.25	avg 1.5	avg 44.25	avg 371.93
				{ 34				
1	2 " daily	12	1.64	7	0	37	44.0	300.38
1	2 " "	16	1.10	9	23	41	47.0	376.40
1	2 " alternate days	16	2.19	16	8	65	30.0	387.28
1	2 " "	16	2.19	16	1	81	107.0	547.26

<sup>1</sup> The *estimated* total weight of both ovaries was obtained by adding the actual weight of the ovarian remnants to the weight of the eggs that had been ovulated (number of eggs times the average weight of a single egg).

<sup>2</sup> Thyroidectomized 4 days before treatment.

<sup>3</sup> In one newt of this series, the 15 glands implanted consisted of 6 from female and 9 from male donors.

<sup>4</sup> Implants in body cavity.

<sup>5</sup> Subcutaneous implants in throat.

<sup>6</sup> Figure computed from data in paper by Adams and Granger, *Proc. Soc. Exp. Biol. and Med.*, 1938, **36**, 552.

7.2 days after the first treatment. The average number of eggs ovulated was 64.9 and laid was 61.5 per newt.

Although only 6 of the newts receiving mouse pituitaries ovulated, in the 8 given 1 AP every other day for 30 days, the average weight of both ovaries (693.65 mg) was nearly twice as great as the average estimated total weight (371.93 mg) of the ovaries of the 4 control newts in this series (Table I). The increase occurred throughout the ovaries since the part containing young oögonia and yolkless oöcytes was 26.90% of the average total ovarian weight in the experimental newts and 25.13% in the controls. A single, large, yolk-laden egg from the experimental and control ovaries weighed the same, 2.42 and 2.41 mg, respectively, but the average number of such large eggs in the former (278.5) was more than twice that in the latter (122.0).

*Rana pipiens* as hosts. (Experiments performed by A.E.A. and B.G.) During December, 1938, and January, 1939, 9 adult female *Rana pipiens* were given fresh anterior pituitaries from adult male mice. The substance was injected in small amounts of distilled water into the body cavity or the dorsal lymph sacs of the host. Six controls received 3 or 4 fresh anterior lobes from *Rana pipiens* females and 4 received fresh mouse brain in amounts similar to those of mouse pituitary. A male injected with frog AP was placed with each female, except 2 given mouse brain, 1 or 2 days after injections began.

It is evident that ovulation did not occur in the 9 frogs given amounts of mouse AP ranging from 35.23 mg (43 APs) to 229.2 mg (269 APs) (Table II). All of them had mature ovaries at autopsy or laparotomy. The 3 which were laparotomized were given a single dose of 4 APs from female frogs (avg, 4.9 mg) 1 or 2 days after laparotomy and all ovulated 3 days later. The 6 controls receiving frog APs (avg dose, 5.37 mg) laid eggs or eggs were pressed from the cloaca on the first or second day after treatment. The 4 frogs given fresh mouse brain (41.31, 88.2, 96.39, 232.0 mg) did not ovulate or appear to suffer any toxic effects from the treatment.

From the experiments in which *Triturus viridescens* was the host, it appears that there is no "zoological specificity" of the gonadotropic hormones of the vertebrate anterior pituitary, for ovulation was secured, but in the series where *Rana pipiens* was the host and no ovulation occurred, the data favor the other concept. However, when previous experiments are considered where negative evidence has been superseded by positive, especially when the dosage has been increased, as in the reaction of toads to fish and frog anterior pitui-

TABLE II.  
*Rana pipiens* Given Anterior Pituitaries from Adult Male Mice.

No. of frogs	Ant. pituitaries		Treatment	Results
	No.	Wt (mg)		
3	43	35.23	5 doses in 6 days (6, 6, 12, 12, 7 APs)	Killed 8th day; no ovulation
11	88	67.9	1 dose	Killed 9th day; no ovulation
1	90	76.02	4 doses in 5 days (36, 18, 18, 18 APs)	Escaped 14th day; no ovulation <sup>2</sup>
1	90	81.69	,, ,,	Laparotomized 14th day; no ovulation <sup>3</sup>
1	100	88.8	1 dose	Laparotomized 7th day; no ovulation <sup>3</sup>
1	108	92.89	3 doses in 5 days (36 APs per dose)	Killed 13th day; no ovulation
1	269	229.2	3 doses in 6 days (100, 100, 69 APs)	Laparotomized 7th day; no ovulation <sup>3</sup>

<sup>1</sup> Splenectomized the day before treatment.

<sup>2</sup> This frog weighed 58.0 g at beginning of treatment. Attempts were made daily to press eggs from cloacal opening without effect. It is probable that its ovaries were mature.

<sup>3</sup> One or two days after laparotomy a single dose of 4 APs from adult female frogs (avg dose 4.9 mg) was administered and the frog ovulated 3 days later.

tary,<sup>8</sup> or of mice to frog anterior pituitary,<sup>5</sup> it seems wise to suspend judgment until further tests are carried out. Perhaps the dosage is not yet sufficient, for Gallien<sup>2</sup> estimated that extract from 1.6 to 3.0 g of fresh beef anterior lobe was necessary to induce ovulation in *Rana temporaria* comparable to that caused by 6 to 9 of its own glands, or only a few milligrams of substance. Moreover, these 2 hosts, *Triturus viridescens* and *Rana pipiens*, vary greatly in their reaction to gonadotropic hormones. The former ovulates readily after anterior pituitary from different species of newts, from *Rana pipiens*, from *Bufo terrestris*, from fowl, and after mammalian anterior pituitary extracts (phyone and hebin)<sup>1</sup> and pregnancy urine (Antuitrin-S),† whereas the latter responds without difficulty to homoplastic and heteroplastic treatment<sup>6</sup> but less easily or negatively to xenoplastic (newt) or dysplastic (fish, mammal) anterior pituitary substance.<sup>7</sup> Similar variations in the reaction to gonadotropic hormones occur in many of the vertebrates—other species and genera of anurans and urodeles, the 2 sexes of amphibians, birds and mammals, and also different mammalian forms (rats, mice, rabbits, guinea pigs).<sup>8</sup>

Even though a positive result was secured with mouse anterior pituitaries certain data indicate that the response occurs with some difficulty. For example, the percentage of newts ovulating is smaller, the number of eggs ovulated or laid per newt is much less, the ovulation occurs much later and the dosage required is greater than in controls given whole pituitaries from newts. It looks as if some barrier prevents as full a response after dysplastic treatment as that which occurs when a homoplastic one is given. Perhaps the release of the active principle of the pituitary substance is slowed due to the differences in the metabolism of warm- and cold-blooded forms or to antagonistic reactions between tissues of host and donor species.<sup>9</sup> However, the results secured in the series of newts given 1 mouse AP

<sup>5</sup> Adams, A. E., and Granger, B., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 585; Adams, A. E., and Tukey, G., *Anat. Rec.*, 1938, **71**, 1 (see Table I); Zwarenstein, H., *Nature*, 1937, **140**, 588.

† We are indebted to Dr. Oliver P. Kamm of Parke, Davis and Co., for the Antuitrin-S.

<sup>6</sup> Rugh, R., *J. Exp. Zool.*, 1935, **71**, 149 (Tables I and II); *Physiol. Zool.*, 1937, **10**, 84; Creaser, C. W., and Sheolnek, M., *Mich. Acad. of Sci., Arts and Letters*, 1938, **24**, 85, Table II.

<sup>7</sup> Adams, A. E., and Granger, B.;<sup>4</sup> Creaser, C. W., and Gorbman, A.;<sup>2</sup> Creaser, C. W., and Sheolnek, M.;<sup>6</sup> Wills, I. A., Riley, G. M., and Stubbs, E. M.<sup>3</sup>

<sup>8</sup> Allen, E., Editor, *Sex and Internal Secretions*, 2nd Edit., 1939, Williams and Wilkins, Chap. IX, XVI.

<sup>9</sup> Loeb, L., *Physiol. Rev.*, 1930, **10**, 547.

every other day for 30 days where marked increase in ovarian size occurred suggest that no immunity was set up in the hosts over this period. Those results and the cases where ovulation did occur (Table I) and those where the reproductive systems of infantile female mice responded to very large doses of *Rana pipiens* AP<sup>5</sup> support the idea that a certain threshold of hormones must be reached if the gonads are to react, especially if the donor is very far removed from the host in the evolutionary scale.

*Summary.* *Triturus viridescens* ovulated extra-seasonally after implants of mouse anterior pituitary averaging 12.6 mg, although the reaction was slower and less complete than that after approximately 0.84 mg of newt whole pituitary substance, or the ovaries increased markedly in size if the above dosage of mouse pituitary was distributed over a long period (30 days). *Rana pipiens*, however, gave no ovulatory response although much larger doses (35.23 to 229.2 mg) of mouse anterior pituitary were given.