

shelter, etc. for the welfare of the slugs prevailed. Under such circumstances one would expect heavier infestations and an increased supply of anemia cases.

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## Induction of Ovulation in Frogs and Toads.

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Experiments on the induction of ovulation in frogs and toads by means of pituitary inoculations and injections of extracts were carried on during the fall of 1930. The results obtained are in essential agreement with those of Houssay, Giusti, and Lascano-Gonzalez<sup>1</sup> and of Kehl<sup>2</sup> on different species of toads and frogs. At the same time it appears possible to explain the seemingly contradictory results in the work of these authors and in the present work without employing the idea of a "zoological specificity" of anterior lobe hormones suggested by Houssay *et al.*

Mature toads (*Bufo vulgaris*) and frogs (*Rana vulgaris*) were inoculated with anterior lobes of pituitaries or injected with extracts† of mammalian pituitary, human placenta, pregnant urine, or with 0.7% NaCl solution. The pituitaries were placed in the dorsal lymph sacs of the animals, while extracts were injected into the dorsal lymph sac, or the muscles of the hind leg, or (rarely) into the body cavity.

*Inoculations of pituitaries into toads and frogs.* I. The first series of experiments consisted of toads inoculated with toad pituitaries (Table 1).

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<sup>1</sup> Houssay, B. A., Giusti, L., et Lascano-Gonzalez, J.-M., *Compt. Rend. Soc. Biol.*, 1929, **102**, 864.

<sup>2</sup> Kehl, R., *Compt. Rend. Soc. Biol.*, 1930, **103**, 744.

† These extracts were prepared by Dr. Wiesner and members of his section of the Animal Breeding Research Department, to whom I wish to express my appreciation.

TABLE I.  
Toads as hosts of toad pituitaries.

Animal	Pituitary inoculations	Period (in days) of inoculations	Findings*
BvT 1	1 whole pit. 3 pars ant.	12	2001 (or more) eggs laid on 12th through 16th day. Autopsy, 49th day: 7 eggs in oviducts, mass of degenerating eggs in uteri.
BvT 3	3 " "	10	1879 eggs laid on 14th and 15th days. Autopsy, 15th day: 14 eggs in oviducts, 9 in body cavity.
BvT 4	6 " "	10	Death from infection. (No egg-laying or ovulation.)
BvT 5	4 " "	3	1463 eggs laid on 4th and 5th days. Autopsy, 8th day: 1 egg in oviduct, none in body cavity.
BT ♀ 1	10 " "	12	1989 eggs laid on 13th day. Autopsy, 42nd day: no eggs in oviducts or in body cavity.

\* In findings, number of day is given in relation to first inoculation.

Of 5 animals receiving toad pituitary inoculations, 4 laid eggs (Table I), demonstrating the potency of the gonad-stimulating hormones of the anterior lobes. The 5th animal died of an infection. The egg-laying occurred without clasping of females by males in 3 cases. In the one instance in which a pair was treated simultaneously, the male clasped the female (BT ♀ 1) on the 4th day after the treatment had begun, continued his hold during inoculations, and only released her after the eggs had been laid. Many of these eggs were fertilized and a considerable number of embryos developed.

II. The second series of experiments consisted of toads inoculated with frog pituitaries or with frog pituitaries followed by toad pituitaries. Frog pituitaries alone did not induce ovulation or egg-laying in toads. This is definitely true in 2 cases each of which received 25 pars anterior in 41 days and as far as actual deposition of eggs is concerned it is so for 2 others (27 pars anterior in 30 days; 19 pars anterior in 30 days). It seems reasonable, though not provable, to believe that ovulation did not occur in either of these instances until toad pituitary inoculations were begun, 3 and 5 days after cessation of frog inoculations. Then 5 pars anterior of toads given in 4 days induced egg-laying in one case, while 6 in 7 days induced ovulation in the other though actual egg-laying did not occur. The ability of the toad pituitaries in relatively small numbers to induce ovulation in toads presents a marked contrast to the ineffectiveness of even large numbers of frog pituitaries.

III. The third series of experiments consisted of 5 toads inoculated with frozen pituitaries of cattle and 1 with whole pituitaries of freshly killed rabbits over periods of 15 to 23 days. Neither pars anterior alone (9, 10, 11 pieces given in periods of 21, 23, 23 days), nor pars anterior followed by posterior lobe (3 pieces of the former, 7 of the latter in 15 days), nor whole gland (5 pituitaries of rabbits in 16 days) caused ovulation in toads. It might be argued that the frozen pituitaries had lost their potency but this possibility is refuted by the fact that at the time of these experiments potent extracts (tested on mice by members of Dr. Wiesner's section) were being made from such glands.

IV. The fourth series of experiments consisted of frogs inoculated with frog pituitaries, frog muscle, or toad pituitaries (Table II).

TABLE II.  
Frogs as hosts of frog pituitaries or frog muscle or toad pituitaries.

Animal	Treatment	Findings*
F ♀ 1	13 pars ant. ( <i>R. vulgaris</i> ) in 17 days	On 6th day (6 pars ant.) some eggs squeezed from cloaca. On 13th day (8 pars ant.), uterine regions dark through body wall. On 16th day (11 pars ant.), 71 (or more) eggs pressed from cloaca. On 19th and 20th days (13 pars ant.), large clumps of eggs (1812) in water. Some eggs were fertilized by ♂ receiving inoculations and developed into embryos. Autopsy, 20th day: few eggs in uteri and body cavity.
F ♀ 3	6 pars ant.	On 5th day (4 pars ant.) uteri dark through body wall.
F ♀ 4	( <i>R. vulgaris</i> ) in 6 days	No egg-laying. Autopsy, 14th day: uteri full of eggs, some eggs in oviducts, 5 in body cavity of F ♀ 3, 13 in that of F ♀ 4.
F ♀ 5	6 muscle	No egg-laying. Autopsy, 11th day: no ovulation.
F ♀ 6	( <i>R. vulgaris</i> ) in 6 days	
F ♀ 2	13 pars ant. ( <i>B. vulgaris</i> ) in 15 days	On 15th day (11 pars ant.), uteri dark through body wall. On 19th day, large mass of eggs (1415) in water. Autopsy, 19th day: both uteri full of eggs, few in oviducts, none in body cavity.
F ♀ 11	11 pars ant. ( <i>B. vulgaris</i> ) in 6 days	On 4th day (5 pars ant.), uteri dark through body wall. No egg-laying. Autopsy, 9th day: uteri full of eggs, none in oviducts, 1 in body cavity.
F ♀ 12	10 pars ant. ( <i>B. vulgaris</i> ) in 5 days	On 2nd day (4 pars ant.), uterine region somewhat swollen. On 4th day (6 pars ant.), uteri dark through body wall. No egg-laying. Autopsy, 8th day: uteri full of eggs, few eggs in oviducts, 5 in body cavity.

\* In findings, number of day is given in relation to first inoculation.

Inoculations of pars anterior of frogs and toads induced ovulation in frogs (6 cases; egg-laying in 2 of these) while muscle was negative in its effects (Table II). The reaction after toad pituitaries is particularly interesting in that frog pituitaries are ineffective in toads.

*Experiments with injections of extracts or of salt solution.* Toads and frogs were given injections of the following substances: (1) An acid extract of human placenta (known as E94, Wiesner<sup>3</sup>) which induces cornification of the vaginal epithelium when injected into immature mice; (2) an aqueous alkaline extract of anterior lobes of cattle pituitaries (known as 119A, Wiesner and Crew,<sup>4</sup> resembling Evans's aqueous alkaline extract) which induces mucification of the vaginal epithelium when injected into immature mice; (3) 0.7% NaCl solution. In addition toads received injections of an extract made from pregnant human urine (known as 198E, Marshall<sup>5</sup>) which induces formation of blood spots and corpora lutea in the ovaries when injected into immature mice.

I. The first series consisted of injections of E94, 119A, and 0.7% NaCl into toads and frogs. E94 in toads (11.25 cc. in 14 days; 43 cc. in 46 days; 57 cc. in 60 days) or in frogs (9 cc. in 5 days; 12.3 cc. in 9 days), 119A in toads (16.65 cc. in 38 days; 17.5 cc. in 20 days; 27.5 cc. in 59 days), 0.7% NaCl in toads (51, 52, 60, 60, 60 cc. in 56, 57, 60, 60, 60 days respectively) or in frogs (9 cc. in 5 days; 12.3 cc. in 9 days) did not cause ovulation. (In 2 toads only immature tissue was found at autopsy.) *But* injections of 119A into frogs (9 cc. in 5 days; 12.3 cc. in 9 days) did induce ovulation. In these 2 cases no egg-laying occurred but that may have been due to the relatively short period of the experiment.

II. The second series consisted of injections of 198E, and of combinations of E94, 119A, 198E, and inoculation of frog pituitaries into toads. No type of injection, *i. e.*, of a single extract (198E: 6 and 8.8 cc. in 28 and 31 days respectively) or of various combinations of extracts (E94 with 119A: 15, 46, 10 cc. of the former with 21.95, 11, 26.65 of the latter in 59, 60, and 61 days respectively; 119A with 198E: 11, 19.5, 27.2 cc. of the former with 0.5, 4.5, 4.5 cc. of the latter in 25, 42, and 58 days respectively) or of extracts with frog pituitary inoculations (15 cc. of E94 with 17.05 cc. of 119A plus 10 pars anterior in 59 days) induced egg-laying or ovulation in toads. Up to the present similar series of experiments have not been carried out on frogs.

These results seem to permit the following interpretation. The pars anterior of frog and toad pituitaries and aqueous alkaline extracts of anterior lobes of mammalian pituitaries contain hormones capable of stimulating frog ovaries to release their eggs, but only the

<sup>3</sup> Wiesner, B. P., *Edinburgh Med. J.*, February, 1930.

<sup>4</sup> Wiesner, B. P., and Crew, F. A. E., *Proc. Roy. Soc. Edinburgh*, 1929, 50, 79.

<sup>5</sup> Marshall, unpublished data.

hormones of the toad cause this reaction in toads. The statement is supported by the facts that ovulation occurred in toads after toad anterior pituitary inoculations (*cf.* Houssay *et al*<sup>1</sup> in toads, *B. arena-rum* and *B. d'Orbigny*) and in frogs (1) after frog pituitary inoculations (*cf.* Wolf<sup>6</sup> in *R. pipiens*), (2) after toad pituitary inoculations (*cf.* Houssay *et al*), and (3) after injections of an aqueous alkaline extract of mammalian anterior lobes (*cf.* Kehl<sup>2</sup> in *Discoglossus pictus*). The negative results in toads after inoculation with frog and mammalian (cattle, rabbit) pituitaries agree with those of Houssay *et al*, who also report negative findings after implants of fish, frog, snake, hen, rat, guinea-pig, dog and beef pituitaries. These authors interpret their results as indicative of a "zoological specificity" of the anterior lobe hormone but to the writer it seems rather to indicate either a lack of sensitivity to potent hormones or a neutralization of these hormones by the body fluids or secretions of the toad. On the other hand, the absence of any reaction on the part of both toads and frogs to placental extracts (E94) argues against the presence of ovulation-inducing hormones in this extract. The salt solution injections merely serve as controls in experimental procedure and indicate that the shock of injecting extraneous fluids will not bring about ovulation. The negative results after injections into toads of the extract made from pregnant urine (198E; *cf.* Houssay *et al*), should be checked by similar experiments on frogs before a lack of potent hormones can be asserted.

Thus on the basis of the present experiments in frogs and toads as well as those of Wolf, Houssay *et al*, and Kehl, and the evidence that urodeles respond to anterior lobe hormones of anurans (ovulation occurs in *Triturus viridescens* after implants of pars anterior of the frog, *R. pipiens*, Adams,<sup>7</sup> and of the toad, *B. terrestris*, Adams<sup>8</sup>) as well as to those of urodeles (Noble and Richards,<sup>9</sup> Adams<sup>10</sup>), the tentative hypothesis is offered that the apparently contradictory results secured in frogs and toads can be better explained as due to a peculiarity of females of the genus *Bufo* in failing to react to gonad-stimulating hormones of pituitaries other than their own rather than to a "zoological specificity" of these hormones. This hypothesis is to be tested by further experimentation.

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<sup>6</sup> Wolf, O. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1929, **26**, 692; *Anat. Rec.*, 1929, **44**, 206.

<sup>7</sup> Adams, A. E., *Anat. Rec.*, 1930, **45**, 250.

<sup>8</sup> Adams, A. E., *Anat. Rec.*, March, 1931.

<sup>9</sup> Noble, G. K., and Richards, L. B., *Am. Mus. Novitates*, 1930, Jan. 9, No. 396; *Anat. Rec.*, 1930, **45**, 274.

<sup>10</sup> Adams, A. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, **27**, 433.