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The Gonad-Stimulating Potency of the Pars Anterior in Normal and Castrated Newts.

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Few experiments have been done which give evidence concerning differences in gonad-stimulating potency of the amphibian pituitary of the two sexes, either in the normal animal or after castration. Although Bardeen[1] reported no difference in pituitaries of males and females of *Rana pipiens,* Rugh[2] discovered that in this species the pituitary of the female is twice as potent as that of the male, and Rostand[3] has found a similar condition in *Rana temporaria* and *R. esculenta.* Using toad material *(Bufo arenarum),* Houssay, Giusti and Lascano-Gonzalez[4] observed no marked variation in potency of the pituitaries of the normal male, castrated male, normal female, and spawning female, and Novelli[5] was unable to demonstrate any difference in pituitaries of males of this species castrated for 30, 60, and 90 days as compared with those of the normal animal.

The criterion by which potency has usually been estimated is the ovulation (or egg-laying) induced in females in the non-breeding season judged by (1) the number of animals ovulating (or depositing eggs) in a definite time or (2) the number of grafts necessary to cause ovulation (or egg-laying) in a series consisting of a definite number of animals. The speed of the extra-seasonal ovulatory response is dependent on (1) the temperature[6] and (2) the period in the interbreeding season in which the tests are being made.[7]

Using *Triturus viridescens* in the non-breeding season (October through early March), the gonad-stimulating potency of anterior pituitaries of normal males and females and of those castrated for 4 days (Series II), 4 weeks (Series I) and 95 to 100 days (Series

1 Bardeen, H. W., Proc. Soc. Exp. Biol. and Med., 1932, **29**, 846.

2 Rugh, R., *Biol. Bull.*, 1934, **66**, 22.

3 Rostand, J., *Compt. rend. Soc. biol.*, 1935, **120**, 336.

4 Houssay, B,. Giusti, L., and Lascano-Gonzalez, J. M., *Compt. rend. Soc. biol.*, 1929, **102**, 864.

5 Novelli, A., *Compt. rend. Soc. biol.*, 1932, **111**, 476.

6 Bellerby, C. W., *Biochem. J.*, 1933, **27**, 2022; Rugh, R., *J. Exp. Zool.*, 1935, **71**, 149.

7 Rugh, R., *Biol. Bull.*, 1934, **66**, 22; *J. Exp. Zool.*, 1935, **71**, 149; Rostand, J., *Compt. rend. Soc. biol.*, 1935, **120**, 336.

TABLE I.

Number of daily homoplastic transplants of pars anterior from normal and castrated *Triturus viridescens* necessary to induce egg-laying in normal females in the non-breeding season. Fifteen hosts were used in every test except in Series I where twelve received grafts from castrated males.

No. of P.A. grafts to induce egg-laying	Number of Hosts Laying Eggs after Grafts, in 3 Series. Series II				Series I				Series III				Series II, I, III			
	♀	♂	♀†	♂†	♀	♂	♀†	♂†	♀	♂	♀†	♂†	♀	♂	♀†	♂†
2	4	2	5	1									4	2	5	1
3	3	5	4	7					5	1	1	2	8	6	5	9
4	3	5	3	3	6	6	6	7	4	4	6	7	13	15	15	17
5	3	3	2	3		3	4	2	2	6	7	5	5	12	13	10
6					2	2	2	1	3	2	1		5	4	3	1
7	1				2	1			1				4	1		
8					1			1		1		1	1	1		2
9							1	1							1	1
10			1	1											1	1
11	1				2	1							3	1		
12						1								1		
13																
14					2	1							2	1		
15							1								1	
16							1			1				1	1	
Total No. hosts	15	15	15	15	15	15	15	12	15	15	15	15	45	45	45	42
Total No. P.A. grafted	62	54	54	60	108	95	96	61	66	85	68	67	236	234	218	188
Av. No. P.A. grafted per animal in Series	4.1	3.6	3.6	4.0	7.2	6.4	6.3	5.0	4.4	5.7 (4.9)*	4.5	4.5	5.24	5.2	4.84	4.48

*Average if newt receiving 16 P.A. is omitted.

†Castrated.

TABLE II.

Number of newts laying eggs after 5 and 6 daily homoplastic transplants of pars anterior from normal and castrated *Triturus viridescens*. Fifteen hosts were used in every test except in Series I where twelve received grafts from castrated males.

		No. of Hosts Laying Eggs after Grafts.															
		Series II				Series I				Series III				Series II, I, III			
		♀	♂	♀†	♂†	♀	♂	♀†	♂†	♀	♂	♀†	♂†	♀	♂	♀†	♂†
5 P.A.	No.	13	15	14	14	6	9	10	9	11	11	14	14	30	35	38	37
	%	86	100	93	93	40	60	66	75	73	73	93	93	67	78	84	88
6 P.A.	No.	13	15	14	14	8	11	12	10	14	13	15	14	35	39	41	38
	%	86	100	93	93	53	73	80	83	93	86	100	93	78	87	91	90.5

†Castrated.

III) was judged by the average number of grafts (one P.A. daily, intramuscularly) necessary to cause egg-laying in a series of 15 normal females (Table I). In Series II, the newts were gonadectomized between November 24 and December 3 and their pituitaries transplanted between November 28 and December 8 at 20°C. In Series I, castrations were performed between September 21 and October 6 and transplants made between October 30 and November 14 at 14°C. In Series III, the gonads were removed between November 15 and December 4 and grafting occurred between February 15 and March 2 at 20°C. The data were also analyzed on the basis of the number of newts in each group and the total number in all 3 groups that had laid eggs on the sixth or seventh days, *i. e.*, after receiving 5 or 6 pars anteriors (Table II).

It is evident (Table I) that there are no consistent or large differences in the ovulation-inducing potencies of pituitaries of castrated and normal newts as judged by the average number of glands necessary to induce ovulation in a series of 15 hosts. Only in Series I and in the combined data of the three series is the trend similar to that found in birds and mammals where the pituitaries of castrates are more potent than those of normal animals[8] and the pituitaries of normal males more potent than those of normal females.[8] In Series I some of the differences are also statistically significant, *e. g.*, castrated male compared with all the other groups and castrated female compared with normal female (barely significant), but this is not true in the combined data.

It is obvious from the table that there are usually one or two refractory hosts that fail to lay eggs until many pituitaries have been transplanted and this increases the average. For this reason, the number and percentage of the series of newts laying eggs after a certain number of grafts were determined. Using this standard (Table II) the data again show the *tendency* of pituitaries of castrates to be somewhat more potent than those of normals (not true of castrated males of Series II) and those of normal males to be slightly more potent than those of females (Series III is an exception). However, neither the differences in average number of pituitaries necessary to induce egg-laying in a series of animals nor those in the number of animals laying eggs after a certain number of grafts are sufficiently large to prove beyond question that significant

8 Allen, E., Edit., Sex and Internal Secretions, 1932; Domm, L. V., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 308, 310; Engle, E. T., *Am. J. Physiol.*, 1929, **88**, 101; Evans, H. M., and Simpson, M. F., *Am. J. Physiol.*, 1929, **89**, 371, 375; Severinghaus, A. E., *Am. J. Physiol.*, 1932, **101**, 309; Smith, P. E., Severinghaus, A. E., and Leonard, S. L., *Anat. Rec.*, 1933, **57**, 177.

variations in gonad-stimulating potency exist in the pituitaries of castrated as compared with normal newts and of males as compared with females. If all of the series had been killed after five or six grafts, thus utilizing the criterion of ovulation rather than egg-laying, the data might have been more conclusive. It is also possible that gonadectomy in the fall of the year when the gonads are already mature may have relatively little effect on the gonad-stimulating hormonal content of the pituitaries as compared with possible effects in the summer when active spermatogenesis and oogenesis are in progress. This should be determined. But even in the fall, the already matured gonad is dependent for maintenance on the pituitary as the effects of hypophysectomy indicate, so that some gonad-stimulating hormone must be present and being released.

One other point emerges from an examination of the tables, namely that temperature affects the ovulatory reaction, for at 20°C., egg-laying occurred earlier, *i. e.*, after a smaller number of grafted pituitaries, than at 14°C. This is in agreement with the findings of Bellerby and Rugh.[9]

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Precipitation and Color Reaction for Ascorbic Acid: Specificity of Acidified Sodium Selenite Solution.

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Certain compounds of selenium, notably selenious acid and its soluble salts, undergo reduction with the formation of free selenium, which appears as a brick-red precipitate or a brick-red or orange-colored colloidal solution. The smaller the quantity of reducing agent, the more the likelihood of the liberated selenium being sufficiently dispersed to form the colloidal state.

The organic compounds of biological significance which display reducing properties are aldehydes, ketones, carbohydrates with a free carbonyl group, polyphenols, thio compounds, including cysteine and glutathione, and ascorbic acid. The thio compounds reduce more readily than carbohydrates with a free carbonyl group, and ascorbic acid may reduce even more readily than thio com-

[9] See footnote 6.