

animal and that this stasis is known to occur with inadequate hormone supply. The graft content of these animals has not yet been studied in comparison with that of the individuals which did not metamorphose.

The thyroids of these animals have the character to be expected from their activity. That of the normal control tigrinum shows higher epithelium and some folding of follicles following the discharge associated with metamorphosis. The control axolotl shows the characteristic low epithelium and well filled follicles. In the axolotls which metamorphosed under the influence of the tigrinum hypophysis, there is some folding of follicles and a moderately high epithelium. The animal with stasis shows low epithelium with some indications of partial discharge.

These experiments present further evidence that the hypophysis of the axolotl is responsible for the non-occurrence of metamorphosis owing to inactivity in the production of the hormone associated with the releasing mechanism of the thyroid. The hypophysis of the axolotl is also less potent in the production of hormones associated with pigmentation and certain growth factors.

### 10360

#### Effect of Pregnancy Urine Extract on Lactation in the Rat.\*

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(Introduced by C. W. Turner.)

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Numerous papers have appeared reporting success in inhibiting lactation by hormone treatment. With the exception of Nelson's<sup>1</sup> work on the guinea pig, these investigations have not indicated whether the inhibitory action was (1) directly on the secretory epithelium of the mammary gland, (2) by the suppression of the secretion of the pituitary hormones necessary for the maintenance of lactation or (3) by the suppression of the release of pituitary hormones required for lactation. Reece and Turner's<sup>2</sup> work indicated

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<sup>1</sup> Nelson, W. O., *Am. J. Anat.*, 1937, **60**, 341.

<sup>2</sup> Reece, R. P., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.*, 1937, 266.

that the inhibitory action of estrogen on lactation in the rat could not be attributed to the suppression of either the secretion or the release of the lactogenic hormone from the pituitary.

We were interested in attempting to determine the route of the inhibitory action of pregnancy urine extracts on lactation, the inhibitory action having been previously demonstrated by others.<sup>3-6</sup> We were unable to inhibit lactation materially with a pregnancy urine extract (Antuitrin-S†) and as a result the work could be carried no further. The results, however, are being recorded because it appears that factors other than hormonal ones may influence the degree of lactation inhibition following the administration of Antuitrin-S.

The first injection into the experimental lactating rats was made shortly after parturition. The number of young in the litters was fixed at 6 and daily weights were recorded. All of the animals used in this experiment were fed the following ration: yellow corn 76%; linseed oil meal 16.0%; crude casein 5.0%; ground alfalfa 2.0%; NaCl 0.5%; and CaCO<sub>3</sub> 0.5%. To this mixture 5% of butter was added after grinding. Fresh whole milk was fed daily and lettuce once each week. Fifty-two lactating rats and their litters were used in this study. The control and experimental lactating rats in the first group were sacrificed on the 22nd day of the lactation period and their pituitaries and ovaries removed and weighed. The lactating rats in the other groups were not sacrificed.

Thirteen rats were injected daily for the first 5 days of the lactation period with 100 r.u. of Antuitrin-S. The first injection was made intraperitoneally and the 4 subsequent injections were made subcutaneously. Ten lactating rats and their litters served as controls. As judged by the rate of growth of the young, there was little evidence, if any, of lactation inhibition in the experimental animals. Following weaning the rate of growth of the pups from the experimental rats was just as rapid as that made by pups nursing control animals, the average weight of the pups in both groups being 65 g when 31 days old. The average weight of the ovaries from the control and experimental lactating rats was 81 mg and 130 mg respectively. With one exception, the ovaries of the injected rats were heavier than those from the non-injected rats. The pituitaries

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<sup>3</sup> Enzmann, E. V., and Pincus, G., *Am. J. Physiol.*, 1933, **103**, 30.

<sup>4</sup> Jongh, S. E. de, *Acta brev. Neerland.*, 1933, **3**, 88.

<sup>5</sup> Selye, H., Collip, J. B., and Thomson, D. L., *Endocrinology*, 1934, **18**, 237.

<sup>6</sup> Cannon, F. E., *Proc. Soc. Exp. Biol. and Med.*, 1937, **37**, 52.

† We are indebted to Dr. Oliver Kamm of Parke, Davis and Company for the Antuitrin-S used in this experiment.

from the non-injected animals averaged 12.3 mg while those from the injected rats averaged 11.6 mg.

Two rats were injected intraperitoneally daily for the first 5 days of the lactation period with 200 r.u. of Antuitrin-S. Two additional rats were given the same treatment except that the extract was injected subcutaneously. Two hundred r.u. of the hormone injected subcutaneously were no more effective than 100 r.u. injected subcutaneously. There was, however, a slight inhibition of lactation in the 2 rats injected intraperitoneally with 200 r.u. of Antuitrin-S.

Three rats were injected intraperitoneally daily for 5 days with 200 r.u. of Antuitrin-S in the morning and this dosage was repeated in the afternoon. At 21 days the pups nursing the injected rats weighed 22% less than did those nursing the control mothers. Inasmuch as a considerable volume of liquid was being injected into the experimental rats it was important to ascertain the influence of this factor on lactation. Three rats were injected intraperitoneally for 5 days with 4 cc of physiological saline in the morning and 4 cc in the afternoon. This treatment caused no inhibition of lactation.

Four rats were injected intraperitoneally daily for 5 days or less with 400 r.u. of Antuitrin-S in the morning and 400 r.u. in the afternoon. The 6 pups of one rat were all dead on the 3rd day and the 6 pups of another rat were all dead on the 4th day. The pups of the other 2 rats weighed 33% less on the 21st day than did those nursing control mothers. It is doubtful, however, if this inhibition of lactation can be attributed to hormonal action only as each injection made the females very sick, the reaction beginning 3 to 4 minutes after the injection. The inhibition was not caused by the volume of liquid injected because an equal volume of physiological saline injected into 2 additional rats elicited no such response.

The above results indicated that the injection of Antuitrin-S during the first 5 days of the lactation period would not markedly inhibit lactation. It seemed worth while to determine the effect of longer periods of injection. Four rats were injected intraperitoneally daily for 16 days with 200 r.u. of Antuitrin-S. On the 15th and 21st day the young of the injected rats weighed 27% less than the young of the control animals. These results are summarized in Table I.

These results are much in contrast to those obtained by Connon,<sup>6</sup> who reported marked inhibition of lactation in the albino rat following Antuitrin-S injections. It may be significant that the control average weight at 21 days reported by Connon (23.2 g) was much lower than our control average figure of 37 g.

TABLE I.  
Effect of Pregnancy Urine Extract on Lactation in the Rat.

No. of Lactating Animals	Daily dosage of Antuitrin-S, r.u.	Route of Injection	No. of young on first day	Avg wt of young on the following day of the lactation cycle, g					No. of young weaned on the 21st day	Avg wt of lactating rats when litters were weaned, g
				1	5	10	15	21		
10	—	I.P. <sup>1</sup>	60	5.3	9.3	16.8	26.0	36.4	58	258
13	100	S.C.	78	5.3	8.4	15.6	24.3	35.4	75	272
2	—	I.P.	12	5.0	8.8	16.5	24.9	35.1	11	216
2	200	S.C.	12	5.0	7.5	12.5	19.7	30.2	11	229
2	200	S.C.	12	5.5	8.9	15.1	24.4	35.5	12	256
3	—	I.P. <sup>2</sup>	18	5.6	9.7	17.1	26.9	40.7	18	273
3	400	I.P. <sup>3</sup>	18	5.6	7.6	13.1	20.5	31.6	18	286
3	NaCl	I.P. <sup>3</sup>	18	5.6	10.0	18.0	27.7	39.5	18	268
3	—	I.P. <sup>4</sup>	18	5.5	9.4	17.8	27.0	37.8	18	247
2	800	I.P. <sup>5</sup>	12	5.6	7.0	12.0	16.9	25.5	12	260
2	NaCl	I.P. <sup>5</sup>	12	5.5	8.7	16.5	25.1	35.5	12	243
3	—	I.P. <sup>6</sup>	18	5.4	9.1	16.7	25.5	36.3	18	248
4	200	I.P. <sup>6</sup>	24	5.2	7.5	13.5	18.7	26.6	24	265

1 I.P. = Intraperitoneal; S.C. = Subcutaneous. Five daily injections, first one intraperitoneal, followed by 4 subcutaneous. One cc of Antuitrin-S contained 100 r.u.  
 2 200 r.u. injected in the morning and again in the afternoon for 5 days.  
 3 2 cc of physiological saline A.M. and P.M. for 5 days.  
 4 400 r.u. A.M. and P.M. for 5 days.  
 5 4 cc of physiological saline A.M. and P.M. for 5 days.  
 6 200 r.u. A.M. for 16 days.

*Conclusions.* The injection of 100 r.u. of Antuitrin-S daily during the first 5 days of the lactation period caused no inhibition of lactation. Larger dosages and dosages over a longer period of time reduced the rate of growth of the young from 22 to 33%. It would appear, therefore, that one cannot markedly inhibit lactation in the rat by injecting Antuitrin-S if the lactation of the control rat is normal.

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**Seasonal Changes in the Testes of the Passerine Bird,  
*Phainopepla nitens lepida.***

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This study was undertaken to determine the histological changes which take place in the testes of the *Phainopepla* during an entire year. It was hoped that such changes which were noted could be correlated in some way with the behavior of the birds.

The collection of *Phainopeplas* for this study was started on November 26, 1937, and has been carried on periodically up to the present time, December, 1938. The gonads were removed while still warm and fixed in Bouin's solution and then carried through the usual paraffin method. They were sectioned and stained, using Delafield's hematoxylin and eosin for some, and Heidenhain's iron hematoxylin for others.

The testes showed a gradual increase in size from a minimum of one mm on November 26, 1937, to their maximum size of 8 mm, which was recorded on May 14, 1938. While the general trend throughout the spring was upward in size some individual variations were noted.

The testes showed no rapid increase in size until March 24th, and few sperms appeared in the lumina of the tubules until May 12th. This is rather unusual for in most seasons on the desert breeding starts in February and is at its height by the end of March. Although the rains were late and it stayed cool much later than usual this year it does not seem that the reproductive cycle should have been slowed to such an extent. Yet this seemed to be the true condition, as it was further substantiated by extensive field excursions during that period.