

# Sex Hormone Response of the Hemipenis of *Eumeces fasciatus* as Reflected by Organ Weight

A. E. REYNOLDS, DePauw University

## I. Introduction

Such studies as have been made on the reproductive physiology of male lizards have demonstrated a number of "target organs" responsive to the sex hormones. Among these are the epididymis and the "sexual segment" of the kidney, which have been shown to undergo, in the normal animal, seasonal and periodic changes correlated with similar changes in the testis (*Anguis fragilis*, Dalcq, 1921; *Lacerta muralis*, Padoa, 1933, Herlant, 1933; *Lacerta agilis*, Regamey, 1935; *Takydromus tachydromoides*, Takewaki and Fukuda, 1935). Further, bilateral castration induced changes in coloration and femoral glands in *L. agilis* (Matthey, 1929), regression in epithelial height and secretory power in both epididymis and "sexual segment" of the kidney in *Takydromus tachydromoides* (Takewaki and Fukuda, 1935), *Lacerta serpa* (Herlant, 1933), *L. muralis* (Padoa, 1933), and *L. agilis* (Regamey, 1935). On the converse side of the picture, Kehl (1938) induced a "sexual segment" condition fully as active as in the male by injection of benzoate of androsterone into the female of *Uromastix acanthinurus* and Noble and Greenberg (1940) found that administration of testosterone propionate maintained the epididymis and vas deferens of *Anolis carolinensis* in the active condition, and developed the "sexual segment" of both sexes. In *Sceloporus*, testosterone propionate produced a marked increase in epididymal tubule diameter and epithelial height (Forbes, 1940, 1941).

The writer, a number of years ago, initiated a program of study on the reproductive physiology of a native American scincoid lizard, *Eumeces fasciatus*, known commonly as the "red-headed skink" (adult male), the "five lined lizard" (adult female), and the "blue-tailed lizard" (immature forms). An initial report (Reynolds, 1943) described the seasonal and cyclic states exhibited by the testis, epididymis, and "sexual segment" of the kidney. Experimental results were also presented showing that the epididymis and "sexual segment" became involute as a result of bilateral castration, and that both could be stimulated by the administration of testosterone propionate. In general, *Eumeces fasciatus* evinced responses consistent with those reported for other lizards as briefly reviewed above.

On *a priori* grounds it would appear that the copulatory sac or hemipenis is another accessory organ vital to the accomplishment of reproduction. As such, its physiological and histological state might logically be expected to parallel that of other accessory reproductive organs by exhibiting seasonal cycles of hypertrophy and involution which may be correlated with seasonal state of the testis. Further, it would be of interest to inquire as to whether or not the hemipenis is under sex

hormone control. Information bearing on these problems is currently at hand in the form of data based on the fresh gross weights of the hemipenial organs concerned, and it is the purpose of this paper to present this information. The anatomical relations of the hemipenis of *Eumeces fasciatus* have been previously described (Reynolds, 1940).

## II. Materials and Procedures

At each sacrifice, organs (including hemipenes) desired for study were measured, weighed, or otherwise described either *in situ* or immediately after removal, prior to being dropped into a histological fixative for treatment and subsequent study. Speed was achieved in weighing freshly-removed organs by the use of a magnetically-damped analytical balance (Christian Becker) of the chainomatic type. Body weights were taken on a triple-beam balance; if not anesthetized the animals were contained in a small silk bag with zipper closure, the weight of the bag being deducted from the total to get the weight of the lizard.

Since both animals and hemipenes differ considerably in actual weight, such differences have been smoothed out by the use of means, and by expressing hemipenis weight as a decimal fraction of body weight. Since the decimal fractions are small, they are converted into larger numbers by multiplying by  $10^4$ . Accordingly, the important index values presented in the Tables below are listed as "Weight Ratios" derived by the formula:

$$\text{Weight Ratio (hemipenis)} = \frac{\text{Mean hemipenis weight}}{\text{Mean body weight}} \times 10^4$$

Any given Weight Ratio may be converted into a decimal fraction of body weight by multiplying by  $10^{-4}$ .

A previous paper (Reynolds, 1943) makes brief mention of techniques employed in administering hormones and in operations.

## III. Results

### A. Males.

1. *Normal Seasonal Effects.* In Table 1, Part A, are presented the data on 28 normal male lizards studied in a seasonal manner and sampling nine months of the year. A seasonal variation in hemipenis weight is clearly indicated by a glance at the column headed "Hemipenis Weight Ratio". Greatest hemipenis weight occurs in May and June, with the maximum apparently occurring in May. Regression is in progress in July, and the period from October through January is apparently one of involution. Seasonal increase in weight is again in evidence by the month of February.

In Table 1, Part B, the first two entries represent a rearrangement of the data on the 28 normal animals. Here, for purposes of comparison, an arbitrary division has been made such that the months of January, July, October, and November are considered as months of "involution", whereas the months of February, March, April, May, and June, are considered as months of "activity". Thus divided into categories, the weight ratios reveal a difference which further supports the thesis of seasonal variations in weight.

TABLE 1. Hemipenis Weight Ratios of Male *Eumeces fasciatus*.

	Number of		Mean Weight		Hemipenis Weight Ratio
	Animals	Hemipenes	Hemipenis (mg)	Body gms)	
A. Seasonally, by Month, for Normal Animals					
January . . .	2	4	18.20	15.55	11.70
February . .	5	10	15.37	6.82	22.50
March . . . .	9	18	13.10	6.10	21.50
April . . . . .	4	8	22.85	14.10	16.20
May . . . . .	1	2	30.80	7.00	44.00
June . . . . .	1	2	23.00	6.80	34.00
July . . . . .	3	6	46.10	30.00	15.40
October . . .	2	4	35.60	27.50	12.90
November . .	1	2	52.75	48.00	10.99
B. Comparison, by Groups, of Various Categories of Animals					
All Normals at involu- tion . . . . .	8	16	37.39	28.01	13.35
All Normals at "activ- ity" . . . . .	20	40	16.99	7.96	21.34
All bilateral castrates . .	5	10	15.78	9.48	16.64
All andro- gen-treated animals . . .	6	11	64.50	22.46	28.71

2. *Effect of Bilateral Castration.* The third entry in Table 1, Part B, consolidates the results on hemipenis weights from five male animals, all sacrificed during "active" months, that had been bilaterally castrate for periods of 27, 47, 184, 459, and 630 days, respectively. It can be seen that the hemipenis weight ratio agrees rather closely with that of normal males at involution.

3. *Effect of Androgen Administration.* Hemipenial weight data from six male lizards that received injections of testosterone propionate ("Oretone", Schering) are consolidated in the fourth and last entry in Table 1, Part B.

These six animals included three normal males that received the androgen as follows: No. 74, 20 daily injections of 0.1 mg plus a 21st injection of 0.09 mg, totaling 2.09 mg; No. 252, seven injections of 0.1 mg each over 12 days, totaling 0.7 mg; No. 179, nine injections of 0.1 mg each over 21 days, totaling 0.9 mg. The respective individual weight ratios of hemipenes of these three males are: 25.1, 40.0, and 23.1. In addition, the group includes No. 238 which experienced a 31-day period of bilateral castration followed by 8 injections of 0.1 mg of testosterone propionate each over 14 days of time, totaling 0.8 mg; also No. 260 which was bilaterally castrate 6 days followed by an injection period of 15 days during which 8 injections of 0.1 mg each were administered. Respective hemipenis weight ratios are 44.1 and 66.4. The sixth lizard in the group was unilaterally castrate for 11 days after which 10.0 mg of androgen was administered at the rate of 0.5 mg per day for 20 days; individual hemipenis weight ratio is 21.9.

Considering the Androgen-treated group as a whole, it can be seen from the last entry in Table 1 that the hemipenis weight ratio of 28.71 is considerably greater than that of either the normal involute or the castrate group, and exceeds that of the normal group at seasonal activity.

#### B. Females

A very interesting feature of the female of *Eumeces fasciatus* is the possession of paired rudimentary hemipenes, which although very small in actual size are similar in anatomical relations to those of the male lizard. Since a certain number of females have been included in two experimental groups, data on these affords some information as to the relative responsiveness of this male structure (in the female) to androgens and estrogens. For the sake of comparison, results on the oviducts are included.

In Table 2, Experiment A, the first entry presents results on two normal females (Nos. 110, 245) which received 43 Rat Units of estrogen (Progynon-B, Schering) in 9 injections (9th of 3 RU) over 11 days. The second entry also concerns two normal females: No. 227 received 0.6 mg of testosterone propionate in 6 equal doses over 15 days; No. 258 received 0.8 mg of the androgen in 8 equal doses over 16 days. The third entry under Experiment A relates to No. 217 which was bilaterally ovariectomized for 14 days then injected with testosterone propionate to the extent of 0.8 mg in 8 injections over 16 days, and to female No. 228 which, beginning 31 days after bilateral ovariectomy, received 0.8 mg of androgen in 8 doses over 15 days. The fourth entry consolidates results on three females (Nos. 262, 263, 216) which were bilaterally ovariectomized for periods of 20, 16, and 30 days respectively. The last entry is based on two control females.

In Experiment B, Table 2, the first entry concerns a single normal female (195) that received the 165 I.U. of estradiol benzoate in 11 daily injections of 15 I.U. each. The second entry concerns two normal female lizards (Nos. 75, 87) that received 0.1 mg of androgen daily for 20 days plus a 21st injection of 0.09 mg, making a total of 2.09 mg. The third entry records a female (No. 192) that was unilaterally ovariectomized for 31 days prior to sacrifice. The fourth entry in Experiment B

records data on two females (Nos. 196, 200) which were unilaterally ovariectomized 10 and 11 days respectively before initial androgen injections; No. 196 received 18 daily injections of 1.0 mg each while No. 200 received 17 daily injections of 0.5 mg each.

TABLE 2. Weight Ratios of Hemipenes and Oviducts of Female *E. fasciatus*\*.

	No. of Animals	Mean Weight			Weight Ratio	
		Body (gms)	Hemipenis (mg)	Oviduct (mg)	Hemipenis	Oviduct
Experiment A						
Normals plus estrogen . . . . .	2	6.5	6.3	53.6	9.7	82.5
Normals plus androgen . . . . .	2	6.85	10.6	53.2	15.5	78.5
Bilaterally ovariectomized plus androgen . . . . .	2	7.65	14.05	56.4	18.4	73.7
Bilaterally Ovariectomized	3	6.83	2.6	12.9	3.81	18.9
Controls . . . . .	2	4.75	1.8	10.4	3.8	22.0

#### Experiment B

Normals plus estrogen . . . . .	1	16.00	4.3	91.9	2.69	57.4
Normals plus androgen . . . . .	2	28.9	31.9	139.6	11.04	48.3
Right unilateral ovariectomy . . . . .	1	19.0	7.4	22.0	3.89	11.58
Right unilateral ovariectomy plus androgen	2	20.75	14.9	180.95	7.18	87.15
Controls . . . . .	2	15.4	4.1	9.9	2.66	6.47

\* The number of individual organs (hemipenes or oviducts) is, for each category, twice that listed for "No. of animals". Weight Ratios are based on individual organs as units.

Examinations of the hemipenis weight ratios in Table 2 shows a low value (3.81, 3.8, 3.89, 2.66) for those animals of both experiments which received no hormone injections (controls, unilateral and bilateral ovariectomies). In the estrogen-injected series, the two animals of Experiment A evince some increase, while the single animal of Experiment B shows again a low value. The most consistent and greatest increase in hemipenis

weight is exhibited by the androgen-injected females in Experiment A, two of which were normals, while the other two were bilaterally ovariectomized. The four androgen-injected females of Experiment B show weight increases, but not of such amplitude or consistency as those of Experiment A.

For comparative purposes, the oviduct weight ratios, calculated in the same manner as those for the hemipenis, are included in Table 2. It can be readily seen that the oviduct responds to androgen by a weight increase, but it increases to a greater extent under the influence of estrogen. On the other hand, the hemipenis in the same female organisms exhibited maximal response in weight increase to androgen.

#### IV. Discussion

The general program of which the observations reported herein are a part will eventually produce supplementary and amplifying information which will include in particular microscopic studies on the histological make-up of the hemipenis and the variations occurring therein under different seasonal and experimental circumstances; also to be included are behavior studies as well as greater numbers of both normal seasonal and experimental cases. Organ weight data on the hemipenis have been accumulated deliberately with the view of utilizing them as a guide in the planning of further studies. It is the purpose of this paper to reduce and consolidate these data in order to indicate possible trends and in particular to ascertain to what extent the hemipenis might possibly exhibit a behavior similar to that described above for such other accessory reproductive structures as the epididymis and the "sexual segment" of the kidney.

The seasonal arrangement of quantitative results in Table 1 support the thesis that the hemipenis is a variable organ which manifests an involute period characterized by low weight extending from October through January, followed by weight increase which is evident in February and which continues until a maximum weight is reached in May; in June the hemipenis is still large although some decrease has occurred, while in July definite regression is in progress. When this seasonal and cyclic behavior of the hemipenis is compared with the similar waxing and waning of other reproductive components of *E. fasciatus* such as the epididymis and the "sexual segment" of the kidney, a close parallelism is made manifest. Since such changes have been shown to be definitely correlated with seasonal changes in the testis (Reynolds, 1943), the seasonal changes in the normal hemipenis are thus strongly suggestive of hormonal control mediated through the testis. Experimental support for this view is to be seen in part from the fact that the weight ratios of 10 hemipenes from 5 lizards that were subjected to total testis ablation evinced values very comparable to those of normal animals during the involute period, even though sacrifice occurred during the seasonally active period. Further, in six male animals to which testosterone propionate was administered hemipenis weight increased, the response being such that the weight ratio of the hemipenes exceeded that of normal animals during seasonal activity. Thus in so far as weight is an index,

the evidence from seasonal changes in the normal male and from both castration and hormone injection indicate that the hemipenis of *E. fasciatus* is under testis hormone control.

An additional test of hemipenial sensitivity to androgen is made possible by the fact that the organ exists as a bilateral rudiment in the adult female of *E. fasciatus*. As might be expected of a small non-functional rudiment, these hemipenes are in normal control animals very light in weight (Weight ratios of 2.66 and 3.8 in Table 2). The administration of androgen, however, causes the weight ratio to increase to values from 7.18 to 18.4, representing virtually a four-fold weight enlargement to a level roughly comparable to that of the normal male at seasonal quiescence (cf. Table 1). Ovarian ablation had no effect on the hemipenis weight in the female, the weight ratio remaining essentially the same as in the normal animal. The question as to whether this "male" organ resident within a normal functional female is responsive to estrogens is of interest. Table 2 records only three females that received estrogen, an it can be seen that some increase was exhibited by the hemipenis in two cases, none in the third. In contrast, the oviduct, a typically "female" organ, gave maximal responses to estrogen although androgen also provoked marked weight increases. Taken as a whole, the experimental data based on the females indicate that the hemipenis is primarily responsive to androgen.

#### V. Summary

1. Expressing hemipenis weight as a decimal fraction of body weight, quantitative results on 28 normal male *Eumeces fasciatus* sampling nine months of the year indicate maximal hemipenial weight during May and June, regression in progress by July, an involute condition from October through January, and thereafter seasonal weight increase which is evident by February.

2. Such seasonal changes in the hemipenis agree quite closely with seasonal variations in the epididymis and "sexual segment" of the kidney, and all may be correlated with the seasonal variations of the testis.

3. Hemipenis weights of five bilaterally castrate lizards are roughly comparable to those of normal males at seasonal involution.

4. Administration of testosterone propionate elicited, in six males, hemipenis weights greater than those of normal males at seasonal hypertrophy.

5. Rudimentary hemipenes in the female of *Eumeces fasciatus* are unaffected by ovariectomy, make a slight response to estrogen, and a more definite response to androgen.

#### VI. Literature Cited

Dalcq, Albert. 1921. Etude de la spermatogenese chez l'Orvet (*Anguis fragilis*, Linn.) Arch. de Biol. **31**:347-452.

Forbes, Thomas R. 1940. Some results of the implantation of pellets of testosterone and estrone in adult male lizards (abstract). Anat. Rec. (Suppl) **76**:22.

———. 1941. Observations on the urogenital anatomy of the adult male lizard, *Sceloporus*, and on the action of implanted pellets of testosterone and of estrone. J. Morph. **68**:31-69.

Herlant, Marc. 1933. Recherches histologiques et experimentales sur les variations cycliques du testicule et des caracteres sexuels chez les reptiles. Arch de Biol. **44**:347-468.

Kehl, R. 1938. Action de l'androsterone sur le "segment sexuel" urinaire de l'Uromastix femelle. Compt. rend. Soc. Biol. **127**:142-144.

Matthey, R. 1929. Caracteres sexuels secondaires du lezard male. Bull. Soc. vaud. Sc. nat. **57**:71-81.

Noble, G. K. and B. Greenberg. 1940. Testosterone propionate, a bisexual hormone in the American chameleon. Proc. Soc. Exp. Biol. Med. **44**:460-462.

Padoa, Emanuelle. 1933. Ricerche sperimentali sui pori femorali e sull' epididimo della lucertola (*Lacerta muralis*, Laur.) considerati come caratteri sessuali secondari. Arch. Ital. di Anat. e di Emb. **31**:205-252.

Regamey, Jean. 1935. Les caracteres sexuels du lezard (*Lacerta agilis*, L.) Rev. Suisse de Zool. **42**:88-168.

Reynolds, Albert E. 1940. Some gross anatomical relations of the male urogenital system and other internal organs in *Eumeces fasciatus*. Proc. Ind. Acad. Sci. **49**:233-242.

\_\_\_\_\_ 1943. The normal seasonal reproductive cycle in the male *Eumeces fasciatus* together with some observations on the effects of castration and hormone administration. J. Morph. **72**:331-377.

Takewaki, Kiyoshi, and Soichi Fukuda. 1935. Effect of gonadectomy and testicular transplantation on the kidney and epididymis of a lizard, *Takydromus tachydromoides*. J. Fac. Sci. Tokyo Imperial Univ. **4**:63-76.