warrant further investigation as to the possible role of these substances on sexual differentiation.

CONCLUSIONS

Although the study of sex determination and, to a lesser extent, sexual differentiation in reptiles have received a great deal of attention during the past decade, more work is needed to fill large gaps in our understanding of these phenomena in reptiles (and other vertebrates as well). For example, reptiles exhibiting TSD are suitable for studies of geographic and interspecific variations in those parameters which affect sex ratios. Such studies would aid in understanding the coevolution of sex determination, reproductive biology, sex ratio, and biogeography. Further areas of interest include: the determination if factors other than temperature influence sex determination; the type(s) of sex determining mechanism(s) found in amphisbeanids and the tuatara; differences in the responses to hormone between species exhibiting TSD and those with GSD; and the search for a species that exhibits both GSD and ESD. This small sample of the issues waiting to be resolved indicates that studies of sex determination and sexual differentiation in reptiles will be fertile areas of inquiry for years to come.

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EFFECT OF EXOGENOUS TESTOSTERONE ON THE EPIDERMAL GLANDS OF *HEMIDACTYLUS FLAVIVIRIDIS*

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ABSTRACT

The influence of exogenous testosterone on the epidermal glands i.e. ß-glands and pre-anal glands, of *Hemidactylus flaviviridis* was studied 15 and 30 days post treatment during breeding and non-breeding phases of gonadal cycle. Parameters like length and breadth of pre-anal glands and their cellular and nuclear dimensions, appearance of further development of ß-glands were considered. In general, it was found that the changes observed 30 days post treatment were more obvious, however, 15 days of treatment did influence both the glands studied, to some extent. Also the exogenous hormone showed pronounced effect during non-breeding period than during the breeding period.

INTRODUCTION

Sexual dimorphism in terms of holocrine epidermal specialization in gekkonines has been confirmed by Maderson and others (Maderson, 1970; Maderson and Chiu, 1970; Menchel and Maderson, 1971, 1975). It has also been observed by Chauhan (1985) that only males of *Hemidactylus flaviviridis* possess the epidermal glands, i.e. pre-anal glands and ß-glands and females do not possess any of these. It was also observed that pre-anal glands' activity varied with respect to testicular cycle (Chauhan and Chauhan, 1985). The pre-anal glands are holocrine structures which open on the ventral femoral side through pores, whereas ß-glands are nothing but glandular cells developed within the epidermal cell layers.

The effects of sex steroids (chiefly the androgen) on the epidermal glands of different gekkonid lizards have been extensively studied (Maderson and Chiu, 1974; Chiu *et al.*, 1970, 1975 and Maderson *et al.*, 1979). Their findings suggested that only males have pre-anal glands and not females. It was also proposed that the differentiation of epidermal glands involved a synergistic action between androgens and hormones responsible for shedding.

There was no such study reported on the influence of androgens in the epidermal glands of a gekkonid lizard, *Hemidactylus flaviviridis*, with the observations during breeding and non-breeding phases separately. Therefore, it was deemed worthwhile to see the influence of exogenous testosterone on the epidermal glands, during breeding and non-breeding phases of the gonadal cycle.

MATERIAL AND METHODS

The adult lizards (*H. flaviviridis*) of both the sexes were obtained twice during the year, once around mid-February and secondly in the beginning of July, i.e. during the breeding and non-breeding periods respectively from a local dealer (Baroda, India). They were maintained on the diet of cockroach-nymphs. Water was provided *ad libitum*. Prior to experimentation, lizards were allowed a week's acclimatization to the cage conditions. Sloughing cycle was followed for every lizard to decide stages of sloughing cycle. It was observed that the interslough period on an average was of 22 days. Lizards were treated on the day after sloughing, i.e. having either stage-0 or stage-1 (for details on stages, see Maderson, 1966) for the purpose of ß-gland studies. The animals of the same weight group (18-20 gms) were than isolated in two groups as follows:

Group 1: Males (*H. flaviviridis*).

Group 2: Females (*H. flaviviridis*).

Each group was made up of seventy animals. Ten lizards were sacrificed at the beginning of experiments and designated as untreated normals at zero day (N°). From the remaining animals, one third of each group, i.e. 20 lizards, served as experimental controls (EXC), 20 lizards as experimentals (EX), and rest 20 as the normal controls (NC). The 'EX' lizards were given intramuscular injections of testosterone propionate (Sigma, st. Louise, USA) (200 μg of testosterone in 1 ml of 0.9 per cent saline), on alternate days, each time 0.05 ml per 20 gms of body weight of hormone was given. 'EXC' animals were injected with 0.05 ml of 0.9 per cent saline only and no hormone per 20 gms of body weight, 'NC' animals were not treated in any manner.

The experimental and control animals were sacrificed at the end of 15 days (EX^{15} and EXC^{15} respectively), and 30 days (EX^{30} and EXC^{30} respectively), under hypothermy.

The gross morphometric observations, i.e. pore diameter, length and breadth of pre-anal glands were made using a micrometer fitted to an occular eyepiece of stereozoom dissection microscope.

The histological preparations for pre-anal glands and ß-glands were obtained as described earlier (Chauhan, 1985). The nuclear and cellular diameters were measured with the help of calibrated occular micrometer at considerably higher magnifications. Student 't' test was performed for all these parameters and 'P' values were derived to record statistical significance. Increase in terms of percentage was calculated for pore diameters. All these results have been recorded in respective tables.

RESULTS

I. Observations During Non-Breeding Phase of Gonadal Cycle:

Group 1:

(a) Pre-anal glands:

The exogenous hormonal treatment for 15 days did not reveal significant alterations in pre-anal gland length, breadth and nuclear diameter (Table 1), however, cell diameter showed considerable change ($P<0.05$). While treatment for 30 days showed statistically significant changes in gland length and diameters of cells and nuclei. It was interesting to note that gland breadth remained unaffected even after treatment for 30 days. Pore diameter increased after 15 and 30 days of treatment (Table 1). Thus the microscopic structure of pre-anal gland proper showed a proliferation due to exogenous hormonal treatment. This was clearly evident, especially in germinal cells (GC) and inner differentiating cells (IDC) (Fig. 2) of treated lizards when compared to normal/control ones (Fig. 1). Mitotic figures were also common in GC and IDC of treated animals (EX^{30}), indicating active proliferation. Eosinophilia of differentiating cells in glands of EX^{30} lizards also increased simultaneously. In short, the pre-anal glands of EX^{30} animals, even during non-breeding phase assumed that state of development which resembled to those of recrudescent phase of gonadal cycle of normal lizards.

(b) ß-glands:

Normally ß-glands in the epidermal layers are found to develop during the breeding period. However, the treatment with testosterone during the non-breeding period resulted in the development of ß-glands. It was observed that the development of ß-glands. It was observed that the development of ß-gland was dose-dependent.

Group 2:

(a) Pre-anal glands:

These are normally absent in this group and the treatment did not allow the development of pre-anal glands.

(b) ß-glands:

ß-glands are also absent in this group, however, exogenous testosterone resulted in the development of ß-glands. It was found that the glandular development was dose-dependent.

II. Observations During Breeding Phase of Gonadal Cycle:

Group 1:

(a) Pre-anal glands:

There was not much difference in the pre-anal glands of normal controls (Fig. 3) and treated ones (Fig. 4), except for the fact that cell diameter showed significant difference. Rest of the parameters namely pre-anal glands' length breadth and nuclear diameters did not show statistically significant changes when compared to those of controls (Table 1).

(b) ß-glands:

ß-glands also did not show any significant variation in the treated animals when compared to the controls.

Group 2:

(a) Pre-anal glands:

As stated earlier, pre-anal glands are absent in this group and could not be induced to develop with exogenous hormone treatment.

(b) ß-glands:

ß-glands are also absent in this group (Fig. 5) but the treatment with testosterone was found to induce their development. The development of ß-glands was dose dependent (Fig. 6 and 7).

Since the development of ß-glands due to exogenous testosterone, in this group was similar during breeding as well as non-breeding periods, illustrations for only during breeding period are provided.

	Non-breeding phase					Breeding phase				
	Length (mm)	Breadth (mm)	Pore diameter (μ)	Nuclear diameter (μ)	Cellular diameter (μ)	Length (mm)	Breadth (mm)	Pore diameter (μ)	Nuclear diameter (μ)	Cellular diameter (μ)
N^0	1.1 ± 0.1	1.0 ± 0.3	170	4.7 ± 0.1	9.1 ± 0.5	3.1 ± 0.6	1.3 ± 0.6	185	6.3 ± 0.6	11.5 ± 0.2
NC^{15}	1.1 ± 0.1	1.1 ± 0.0	173	4.8 ± 0.1	9.3 ± 0.4	3.1 ± 0.4	1.3 ± 0.7	186	6.8 ± 0.8	11.6 ± 0.5
EXC^{15}	1.2 ± 0.5	1.1 ± 0.1	173	4.7 ± 0.3	9.3 ± 0.1	3.2 ± 0.7	1.4 ± 0.5	185	7.0 ± 0.6	11.8 ± 0.4
EX^{15}	1.2 ± 0.0	1.1 ± 0.1	174	4.9 ± 0.0	10.2 ± 0.1	3.2 ± 0.3	1.5 ± 0.0	188	6.9 ± 0.4	11.9 ± 0.4
Significant at the level*	NS	NS	2.35**	(P<0.10) NS	P<0.05	NS	NS	1.62**	NS	NS
NC^{30}	1.3 ± 0.3	1.2 ± 0.4	178	4.8 ± 0.5	9.3 ± 0.3	3.2 ± 0.5	1.5 ± 0.4	189	7.0 ± 0.5	11.9 ± 0.0
EXC^{30}	1.3 ± 0.2	1.2 ± 0.0	177	4.8 ± 0.6	9.4 ± 0.0	3.2 ± 0.6	1.5 ± 0.6	188	7.3 ± 0.1	11.9 ± 0.3
EX^{30}	1.8 ± 0.3	1.4 ± 0.1	180	5.4 ± 0.0	10.7 ± 0.2	3.3 ± 0.1	1.6 ± 0.1	190	7.6 ± 0.3	12.7 ± 0.4
Significant at the level*	P<0.05	NS	5.88**	P<0.001	P<0.01	NS	NS	2.70**	(P<0.10) NS	P<0.05

* P values refer to differences between N^0 and EX^{15}; N^0 and EX^{30} periods. The student's 't' test was used to analyze differences in means. NS means non-significant (i.e. P<0.05).

** Depicts per cent increase, values of EX^{15} and EX^{30} compared with N^0.

TABLE 1: Effects of exogenous treatment of testosterone in various components of pre-anal glands of male *Hamidactylus flaviviridis*. Mean value ± S.E.M.

DISCUSSION

The present observations explain the involvement of an androgen (testosterone) in the development of epidermal glands in gekkonid lizards and its effect is dose-dependent. The development of ß-glands in case of females and also in males during the non-breeding period with hormone treatment is suggestive of the fact that the germinal epithelium of these scales (epidermis) respond to androgens by producing glandular elements and this development is dose-dependent. This kind of response could be better expressed in presence of sufficient androgenic stimulus.

The development of ß-glands but not the pre-anal glands in case of treated females is suggestive of the fact that the ß-glands are more responsive than pre-anal glands to androgens. Thus, it seems that germinal epithelium of epidermal layers responded to the androgenic stimulus but not that of pre-anal glands. Probably, the germinal layer of pre-anal gland would respond, if optimum levels of androgens for enough duration are maintained.

In the males, the pronounced effect of exogenous testosterone was evident only during non-breeding period, while during breeding period it seemingly had no effect. It could be realized from these observations that beyond certain level, testosterone exerts no additive influence either due to a feedback mechanism or due to a specific maximum threshold sensitivity of these target organs to such sex steroids. Thus, a direct effect of exogenous testosterone on epidermal glands appears to support the views expressed by Maderson, Chiu and others (Maderson and Chiu, 1970, 1981; Maderson *et al.*, 1977, 1979).

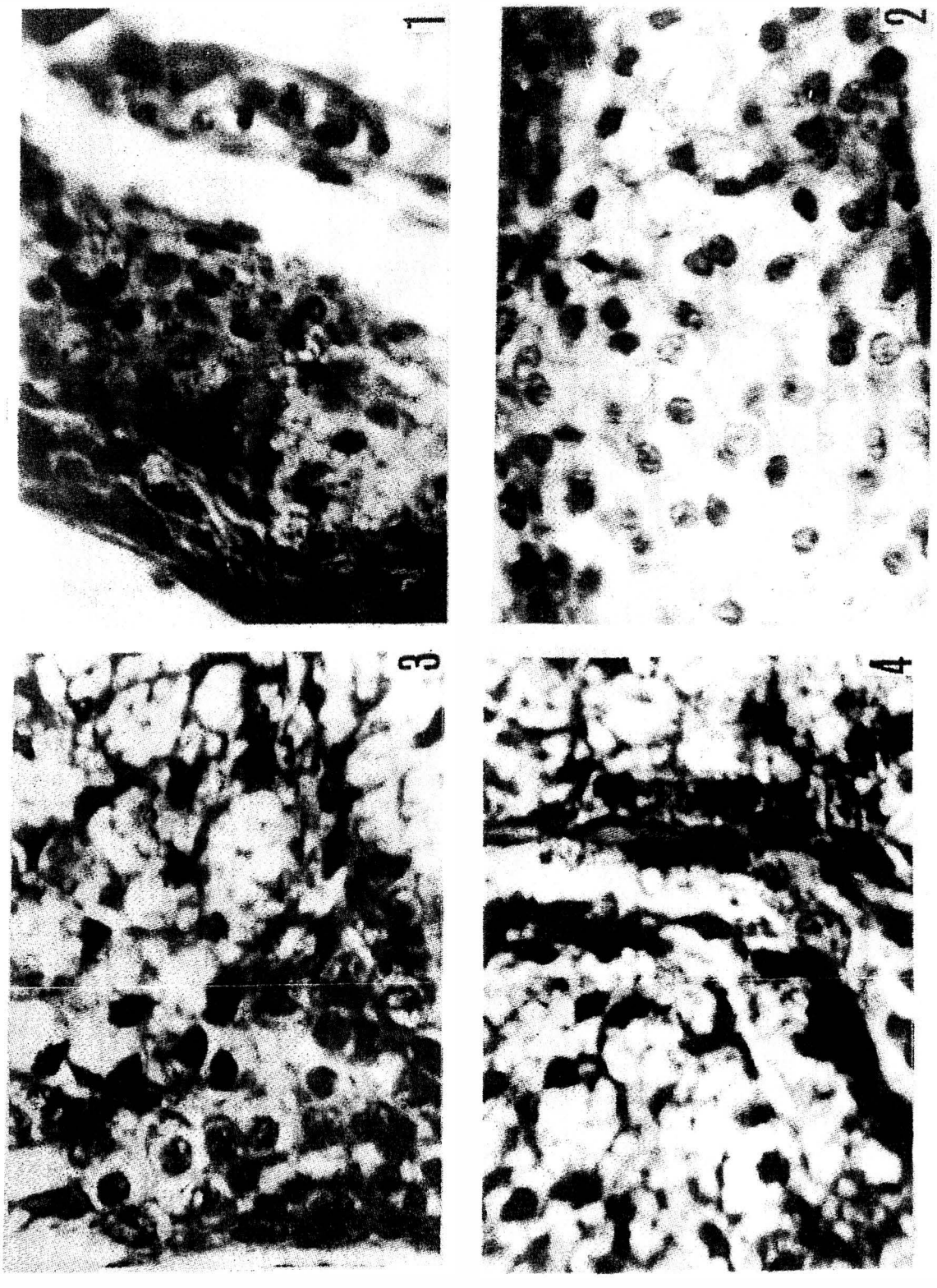

PLATE I Photomicrographs of the pre-anal glands of male *H. flaviviridis*

Fig. 1 Pre-anal gland of normal lizard during non-breeding period.
Note smaller cells with healthy nuclei; gland as such was also of smaller size.

Fig. 2 Pre-anal gland of testosterone treated (for 30 days) lizards during non-breeding period.
Note increase in cell size and glandular mass. Some of the nuclei are unhealthy and cells having them are very large. Gland shows characters similar to that of breeding phase.

Fig. 3 Pre-anal gland of normal lizard during breeding period.
Note the hypertrophy of the gland. Compare it with Fig. 2.

Fig. 4 Pre-anal gland of testosterone treated (for 30 days) lizards during breeding period.
Note that the gland structure does not differ much than that observed during breeding period of untreated lizards, i.e. Fig. 3.

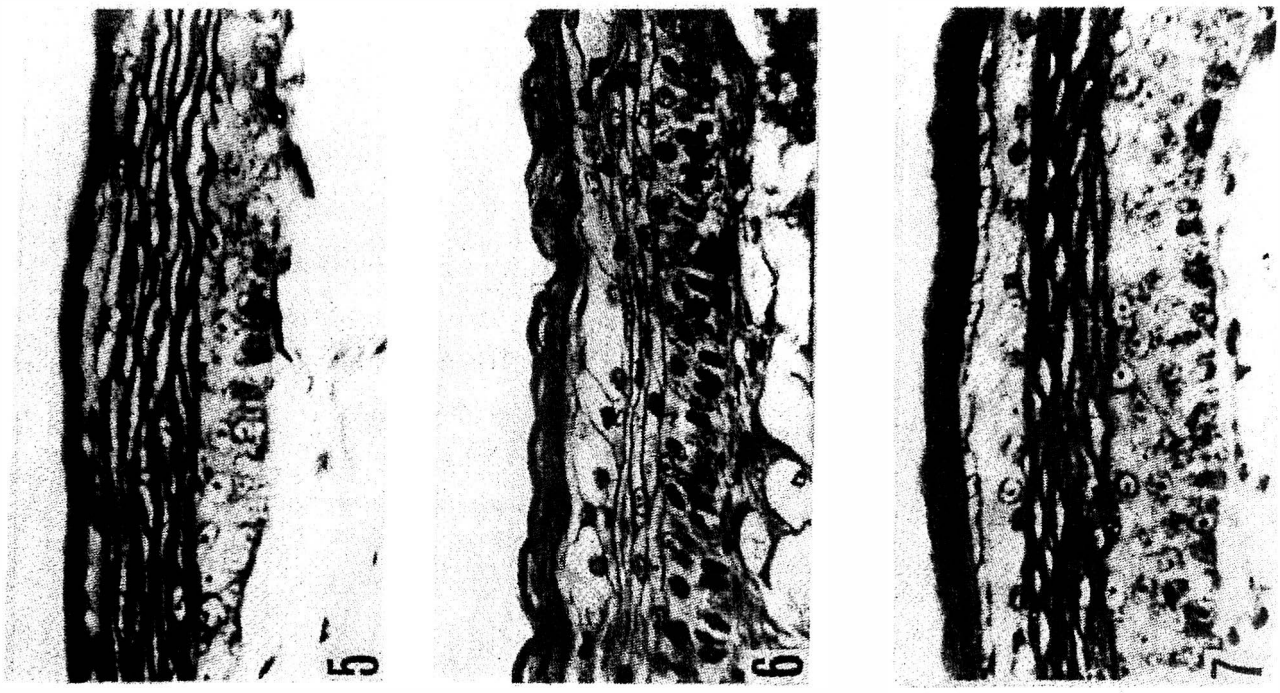

PLATE 2 Photomicrographs of the epidermis of female *H. flaviviridis*.

Fig. 5 Epidermis of the untreated (normal) lizard (decapitated after 30 days, along with treated lizards for 30 days). Note the abscence of ß-gland, but epidermis is in stage-4 of sloughing cycle.

Fig. 6 Epidermis after 15 days of testosterone treatment. Note moderately developed ß-gland.

Fig. 7 Epidermis after 30 days of testosterone treatment. Note well developed ß-gland.

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