STEROIDS IN THE INTERRENAL AND GONADS OF SOME AMPHIBIANS AND CALOTES—A CHROMATOGRAPHIC STUDY

BY S. KASINATHAN AND S. L. BASU

(Department of Biology, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry-605006, India)

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ABSTRACT

The gonadal and interrenal steroid of Rana hexadactyla, Rana cyanophlyctis, Rhacophorus maculatus and Calotes versicolor have been analysed by paper chromatography. In the interrenal of both the sexes of three species of frogs studied, aldosterone and corticosterone could be detected. In the testes of all the three species of frogs studied, testosterone was significantly absent; progesterone and 17β estradiol were detected. In the ovary 17β estradiol, estriol and progesterone were identified. In the adrenals of Calotes, as in frogs, corticosterone and aldosterone were noticed. In the testis testosterone and 17β estradiol were detected; in the ovary progesterone, estriol, estrone and 17β estradiol were found. The significance of the presence of testosterone and the absence of progesterone in the testes is discussed in the light of biosynthetic pathway known in other forms.

INTRODUCTION

Information regarding the nature of steroids in the gonadal and interrenal tissues of poikilothermic animals is scanty. In the interrenal of Rana catesbeiana, Carstensen et al. (1961) have reported the presence of aldosterone and corticosterone. Dale (1962) reported the presence of 17β estradiol in the larva of Rana pipiens and Gallien et al. (1960) isolated 17β estradiol and estrone from the ovaries of Xenopus laevis by paper chromatography.

In the testes of Bufo vulgaris, Chieffi and Lupo (1961 a) have reported the presence of progesterone and 17β estradiol. These authors (1961 b) have also identified the presence of 17β estradiol, estrone and estriol from the Bidder's organ and also from the ovary of Bufo vulgaris. Della
Corte and Cosenza (1965) have reported the same steroids in *Triturus cristatus*.

In reptilia, Gist and de Roos (1964) extracted corticosterone and aldosterone from the interrenal of *Alligator mississippiensis*. Gottfried et al. (1967) by thin layer and gas chromatographic analysis isolated from cobra adrenals, cortisol, corticosterone, aldosterone, testosterone, 18 hydroxy-corticosterone, dehydro-epiandrosterone and estrone. The pattern of testicular steroids obtained from these animals suggested the presence of dehydroepi-androsterone, androsterone 17α hydroxy pregnenolone and estrone. Besides these, no other information is available on the gonadal steroid analysis in reptiles.

In the present investigation, the gonadal and interrenal steroid hormones of 3 species, of frogs, viz., *Rana hexadactyla*, *Rana cyanophlyctis*, *Rhacophorus maculatus* and the garden lizard, *Calotes versicolor* are reported.

**MATERIAL AND METHODS**

The tissues (interrenal, testes and ovaries) were collected during the breeding season and homogenised in aqueous methanol. The homogenate was dialysed for 24 hrs. in a beaker containing 40% methanol. The extract in methanol was dried *in vacuo* and used for chromatography. Paper chromatography of steroids was used adopting the procedure of Barton *et al.* (1952), Burton *et al.* (1951) and Zaffaroni *et al.* (1950).

**RESULTS AND DISCUSSION**

The results of these investigations are summarised in Table I.

Estradiol 17β is found in the testes and ovary in all the three species of frogs and *Calotes*.

Testosterone has been detected in the testes only of the *Calotes* and is lacking in frogs.

Progesterone occurs in the testes and ovary of the three species of frogs and in the ovary alone of *Calotes*.

Aldosterone and corticosterone are found in the interrenal of the three species of frogs and *calotes* but not in the gonads of these animals.

Cortisol could not be detected either in gonad or interrenal tissues in frogs and *Calotes*.

In the present study, it is observed that when testosterone is present progesterone is lacking and *vice versa*. In *Rana catesbeiana*, it has been
TABLE 1

Interrenal and gonadal steroids in some species of Amphibia and Calotes

<table>
<thead>
<tr>
<th>Name of the animal</th>
<th>Organs studied</th>
<th>C\textsubscript{21}</th>
<th>C\textsubscript{19}</th>
<th>C\textsubscript{18}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Corticosterone</td>
<td>Aldosterone</td>
<td>Progesterone</td>
</tr>
<tr>
<td><strong>Amphibia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rana hexadactyla</em></td>
<td>Interrenal</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Testis</td>
<td>-</td>
<td>=</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Ovary</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Rana Cyanophlyctis</em></td>
<td>Interrenal</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Testis</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Ovary</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Rhacophorus maculatus</em></td>
<td>Interrenal</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Testis</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Ovary</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>Reptilia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Calotes versicolor</em></td>
<td>Adrenal</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Testis</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Ovary</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+ Indicates presence; − indicates not detectable.

shown in (Dale and Dorfman, 1967) in vivo and in vitro studies that testosterone could be detected in the testes incubated with radiolabelled progesterone/4-C¹⁴ Ozon (1966) has observed that testosterone is synthesized from progesterone in the testes of *Pleurodeles waltlii*. The role of progesterone as a precursor in the biosynthesis of gonadal androgens was reported by Slaunwhite and Samuels (1956). Biosynthetic pathways of testosterone in gonadal tissues, in elasmobranch (Simpson *et al.*, 1964); and teleostei (Arai *et al.*, 1964) has been described. The present study therefore points to the possibility of testosterone being produced as a metabolic product from progesterone. It is evident that the biosynthesis of C\textsubscript{19} steroids from C\textsubscript{21} steroids in these species proceeds in a similar manner to what has been observed in other vertebrate classes. The chief finding is that progesterone appears to be a precursor in the biosynthesis of testosterone, being utilised as and when needed.

Estrone, estriol and 17 β estradiol have been identified by earlier workers in amphibian ovary (Chieffi and Lupo, 1961, 1961 *a, b*, 1963; Chieffi, 1962). However, we failed to detect the presence of estrone in all the three species of frogs studied by us.
It has been shown that in lower vertebrates, estrogenic steroids may play a vital role in reproductive behaviour being responsible for several modifications of the genital tract during sexual cycle. The significance of progesterone in the ovaries has been correlated with the formation of pre-ovulatatory copora lutea (Chieffi, 1961). The present study also indicates this. It has been experimentally proved (Chieffi and Lupo, 1963) that in Bufo vulgaris estrogens may prevent the atrophy of oviduct after ovariectomy, while secretion from oviducal glands is caused by progesterone or prolactin and not by estrogen. Progesterone apparently plays a similar important role in the physiology of reproduction of frogs studied.

In the testes of Calotes versicolor testosterone and 17β estradiol could be detected while progesterone could not be traced. But in frogs, testosterone is absent in the testes whereas progesterone could be detected. Though testosterone and progesterone picture appears to be different in frog and Calotes, yet in these animals also, biosynthetic pathway appears to be similar to what has been reported in other vertebrates and that progesterone may be a precursor in both cases. It has been pointed out by Gottfried et al., 1967) that the testicular steroids of lower vertebrates may depend, in part, upon the seasonal variations of the enzymes available for substrate conversion within the testes.

Progesterone, 17β estradiol, estrone and estriol were detected in the ovary of Calotes. Corticosterone and aldosterone were present in the adrenals of both the sexes of Calotes. The presence of progesterone and estrone in female indicates their role in the physiology of reproduction in lower vertebrates generally.

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