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

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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 17 β estradiol were detected. In the ovary 17 β estradiol. estriol and progesterone were identified. In the adrenals of *Calotes*, as in frogs, corticosterone and aldosterone were noticed. In the testis testosterone and 17 β estradiol were detected; in the ovary progesterone, estriol, estrone and 17 β 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 17 β estradiol in the larva of *Rana pipiens* and Gallien *et al.* (1960) isolated 17 β 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 17 β estradiol. These authors (1961 *b*) have also identified the presence of 17 β 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 mississipiensis*. Gottfried *et al.* (1967) by thin layer and gas chromatographic analysis isolated from cobra adrenals, cortisone, 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 17 α hydroxy pregnenolone and estrone. Besidse 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 17 β 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 intrerrenal 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 intrerrenal 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 I

Interrenal and gonadal steroids in some species of Amphibia and Calotes

Name of the animal	Organs studied	C_{21}		C_{19}			C_{18}	
		Corticosterone	Aldosterone	Progesterone	Testosterone	Estradiol 17β	Estriol	Estrone
Amphibia								
Rana hexadactyla	Interrenal	+	+	–	–	–	–	–
	Testis	–	=	+	–	+	–	–
	Ovary	–	–	+	–	+	+	–
Rana Cyanophlyctis	Interrenal	+	+	–	–	–	–	–
	Testis	–	–	+	–	+	–	–
	Ovary	–	–	+	–	+	+	–
Rhacophorus maculatus	Interrenal	+	+	–	–	–	–	–
	Testis	–	–	+	–	+	–	–
	Ovary	–	–	+	–	+	+	–
Reptilia								
Calotes versicolor	Adrenal	+	+	–	–	–	–	–
	Testis	–	–	–	+	+	–	–
	Ovary	–	–	+	–	+	+	+

+ Indicates presence ; — indicates not detectabel.

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-C^{14} 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_{19} steroids from C_{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 presenec 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-ovulatory 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 17 β 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, 17 β 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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