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Modification of Sex Differentiation by Steroid Hormones in a Tree Frog (*Pseudacris nigrita triseriata Wied*).* (23687)

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Amphibians have been used most successfully in experiments on hormonal modification of the processes of sex differentiation. While results obtained with different taxonomic orders and families first seemed irregular or even contradictory, accumulation of a considerable body of data is now suggesting a correlation between genic sex type and mode of response(1). In view of the possible bearing of this relationship on the theory of gene action, verification by further extension of the experiments to still untested groups is desirable. The present report on a tree frog bears on a family (hylidae) which, according to Yosida (2) and the senior author's own observations, seems chromosomally related to the family of the common frogs (ranidae).

Material and methods. Normally deposited eggs were collected near Iowa City and kept in finger bowls until hatching of the tiny larvae. On the day when feeding started, groups were separated according to the planned hormone treatments. The experimental lots were placed and reared in steroid solutions of the concentrations indicated in Table I. Mortality was moderate, too low to affect the outcome of the tests.

Results. Testosterone propionate is completely masculinizing the genetically female half of the treated groups. The used concentrations of 50 and 500 μg/l obviously are not limit values, but they are sufficiently apart to permit the conclusion that all effective dosages are masculinizing. The study of normal sex differentiation of the same species by Deal (3) revealed that all male gonads are invaded by numerous pigment cells during and immediately following initial sex differentiation (larvae 15 to 18 mm). They move along with ingrowing mesonephric blastema cells. Ovaries never are pigmented. In the experimental transformation of genetic females into males, in the present experiments, the testes became as darkly pigmented as those of genetic males. In contrast to the persistently masculinizing action of testosterone, the effect of estradiol (and estrone) treatments depends on the dosage level; it is feminization at 50 μg/l, but an almost normal sex ratio at the higher level (Table I).

Discussion. The described experiments with treefrogs give results that are essentially identical with those obtained in more extensive tests with *Rana sylvatica*(4). The slowly but steadily accumulating data on reactions of amphibians belonging to various taxonomic orders and families permit now to distinguish

TABLE I. Effect of Continuous Steroid Hormone Administrations on Sex Differentiation in Pseudacris. Treatments started after closure of gill sacs (Stage 25). All animals preserved and sexed at end of metamorphosis (Stage 33).

Hormone		Females	Intersexes	Males
Testosterone	50 μg/l			27
"	500 "			209
Estradiol	50 "	16		
Estradiol + Estrone	100 " 200 "	104	1	96
Controls		37		33

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2 reaction types (for detailed bibliography consult ref. 1). In the first type, androgens produce complete and durable masculinization of genetically female embryos; gynogens are feminizing at low effective dosages—apparently effecting only an unstable sex inversion of genic males—but at high dosages they are masculinizing genic females. To this type belonged, so far, only the ranids; they are now joined by the hylids. In the second reaction type, represented by several families of primitive anurans (discoglossids, xenopins) and all urodeles, androgens produce either no major effects or, in some instances, they feminize genic males; gynogens however are feminizing the larvae of male genic constitution.

Experiments with the common toad indicate that in general bufonids follow the second reaction type. However, feminization by gynogens is unstable(5), so that in this regard they seem related to the first type. Their intermediary behavior is of some interest in view of the taxonomic position of the bufonids between the higher and the primitive families of the anurans.

Breeding experiments with various sex races and with sex reversed animals so far have shown that in frogs the males are heterozygous for sex determining genes, while in toads, all lower anurans, and salamanders the females are the heterozygous sex. The number of fully investigated species is still small, but all cases conform to the rule that the first reaction type (to steroids) is exhibited by the species with male heterozygosity and the second by those with female heterozygosity. The remarkable parallelism points to some characteristic difference in the mechanisms of gene action in the two types.

Summary. Testosterone masculinizes the course of gonad differentiation in genetically female larvae of treefrogs. Gynogenic steroids are only partially feminizing the gonadal development of genetic males. These results establish for hylids and ranids a common reaction type to steroid treatments, which differs characteristically from that of all other amphibian families which, so far, were submitted to tests.

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Copulatory Reflex by Progesterone and Desoxycorticosterone Acetate.* (23688)

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Using the persistent estrous rat as a test animal, it has been shown that progesterone (1) and desoxycorticosterone acetate (DOCA)(2) are equally effective in restoring estrous cycles. Other earlier reports(3,4,5) had established that DOCA is 1/10 to 1/15 as potent as progesterone in a variety of tests. Most recently using the elicitation of the copulatory reflex in guinea pigs, DOCA was found to be ¼ as potent as progesterone(6). Studies in progress in our laboratory for several years have indicated that the relative activity of these 2 substances in dependent upon the absolute amount used in the test, and have lead to this report.

Methods. Young, adult, female guinea pigs were ovariectomized and allowed to recover for 30 days. The completeness of the surgery was attested to by examination of the extirpated ovaries, lack of estrous behavior in untreated animals, and examination of the

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