

RESPONSE OF GONADS OF FROG LARVAE
TO CHORIONIC GONADOTROPINS AND
SYNTHETIC ANDROGENS¹

CHARLES L. FOOTE

Southern Illinois University, Carbondale

The effects of hormones on the gonads and gonoducts of amphibians have been described numerous times during recent years. The many kinds of experimental animals and the variety of hormonal substances administered have given somewhat inconclusive results (reviews by Blair, 1946; Gallien, 1950). Administering chorionic gonadotropins to adult amphibians in tests for pregnancy in the human being has become a useful and commonplace procedure (Robbins, Parker, and Bianco, 1947; Robbins and Parker, 1948; Galli-Manini, 1948; Wiltberger and Miller, 1948). However, it is reported that administering chorionic gonadotropins to larval anurans has no apparent effect on gonads (Krichesky, 1934; Mintz, Foote and Witschi, 1945). Puckett (1939) reports that precocious development of the gonads of *Rana catesbiana* was obtained with mammalian pituitary extracts, and Blair (1946) found that chorionic gonadotropins administered to juvenile *Bufo fowleri* retarded spermatogenesis and hypertrophied the interstitial tissue of the testis and caused accelerated growth of oocytes in the ovary.

MATERIALS AND METHODS

Larvae of *Rana sphenoccephala* were obtained from a mine pond in

late May. The tadpoles at that time measured between 35-50 mm. total length, with an average length for 13 animals of 44.0 mm. Histological examination of gonads showed that the sex of many of the larvae could not be definitely determined as the gonads were in an immature stage (figs. 1 and 2).

A single injection of 0.025 mgs. of testosterone propionate (Perandren-Ciba)² was given, in sesame oil, to one group of 50 larvae (Group I). One month later a second group of 16 larvae, in which the sex had differentiated, were injected with a like amount of testosterone propionate. Larvae of this group (Group II) were selected from a reserve of control animals which had received no treatment with hormones. A third group of larvae (Group III) received oil only and served as controls.

As the animals began to metamorphose approximately two months after the experiment was started, some of each group were given two daily injections of 0.1 cc. (100 I.U.) of chorionic gonadotropic hormone ("APL"-Ayerst)³ and were then sacrificed at the end of the second day. Some larvae received similar injections of chorionic gonadotropin

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³The chorionic gonadotropic hormone was supplied by Ayerst, McKenna and Harrison, Rouses Point, New York.

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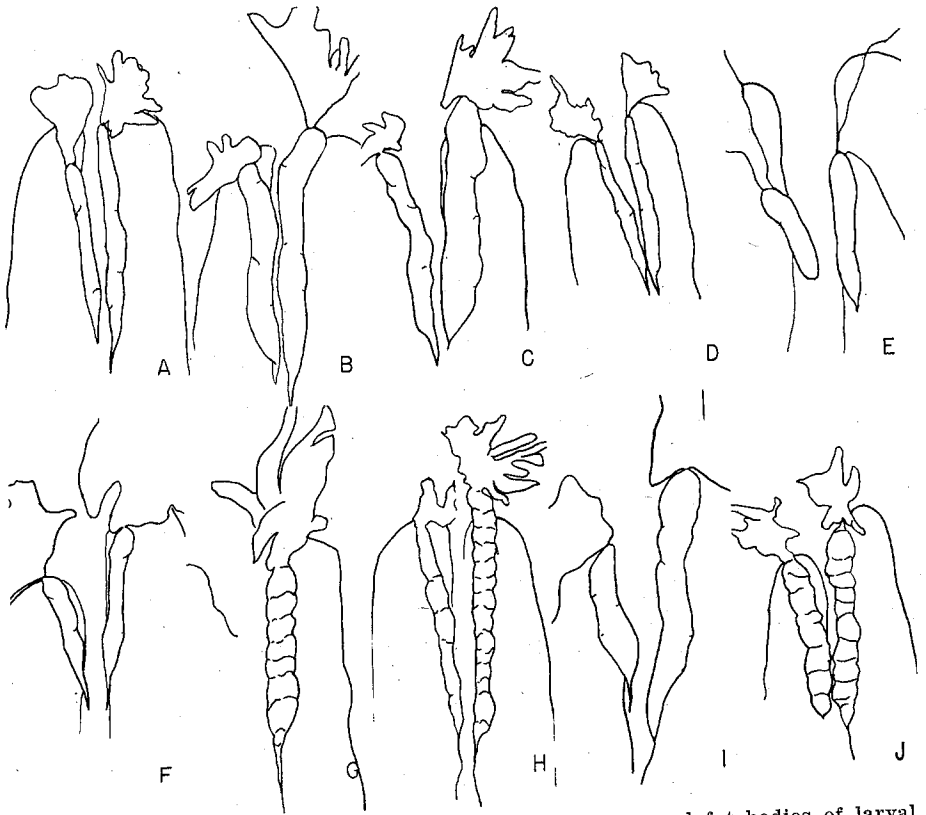


FIG. 1.—Camera lucida drawings of gonads, kidneys, and fat bodies of larval controls (A, male, B, female), gonadotropin-injected larvae (C, female; D, intersex), testosterone-treated larvae (E, F, males), testosterone- and gonadotropin-treated larvae (G, H, I, J, intersexes). X9

two days prior to sacrifice. It had been hoped that metamorphosed animals might be kept for longer periods of time, but the mortality in this group was so high that all animals were sacrificed soon after metamorphosis.

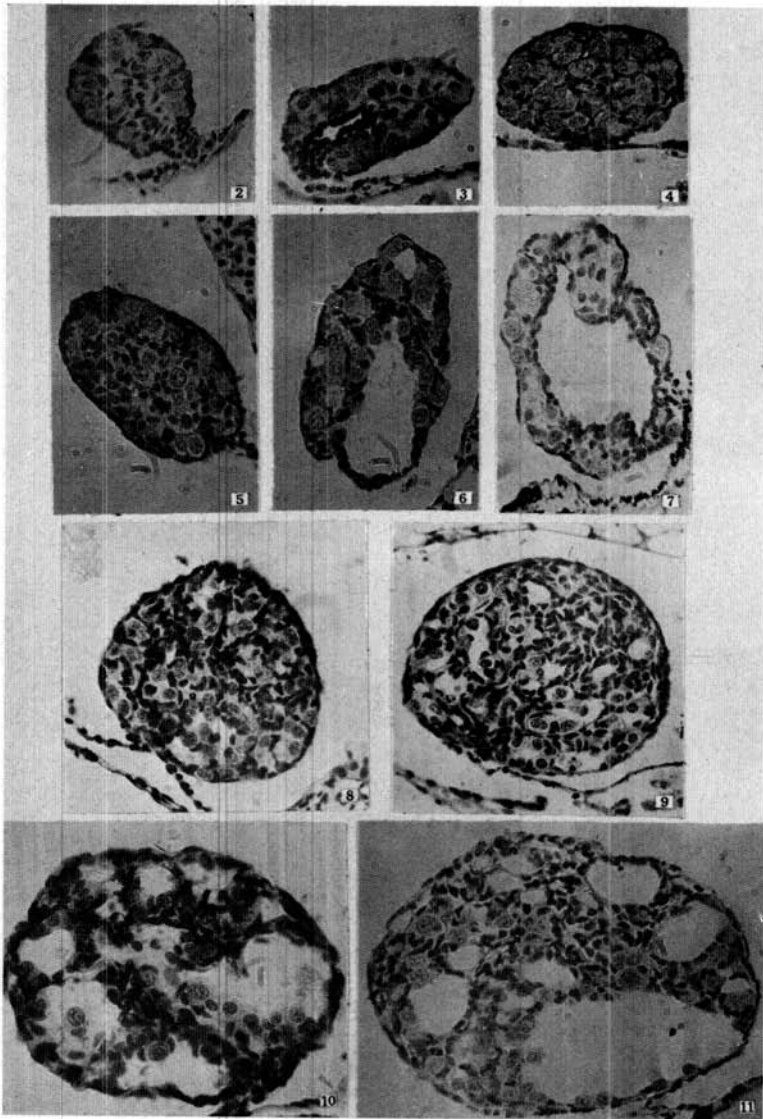
Larvae were kept in well-aerated aquariums in a room where the temperature varied between 15-20° C. They were fed daily on fresh lettuce leaves and dry brewer's yeast.

At the time of sacrifice the abdominal cavity was opened and the animals placed in Bouin's solution.

Serial sections of 10 microns were cut and the tissues stained in Harris's hematoxylin with an eosin counterstain.

RESULTS

As would have been expected from previous investigations the animals of Group I, which received testosterone propionate, had gonads of the male type (fig. 5), as contrasted to the oil-treated controls of Group III which showed a sex distribution of 5 males and 8 females (table I). In Group II, however, where testoster-



FIGS. 2-11.—(2) Ovary of control larva at beginning of experiment. (3) Control ovary showing secondary gonad cavity one month after beginning of experiment. (4) Testis of control larva one month after beginning of experiment. (5) Testis of larva treated with testosterone propionate. (6) Intersexual gonad of larva treated with testosterone propionate and chorionic gonadotropin. (7) Ovary of control larva treated with chorionic gonadotropin. (8) Testis of metamorphosed control. (9) Testis of metamorphosed animal treated with testosterone propionate. (10) Testis of metamorphosed animal treated with chorionic gonadotropin. (11) Testis of metamorphosed animal treated with testosterone and chorionic gonadotropin. X 130.

TABLE I.—SEX DISTRIBUTION IN CONTROL AND HORMONE TREATED ANIMALS.

Groups	Number	Treat- ment	Males	Females	Inter- sexes	Undiffer- entiated	Totals		
							larvae	metamor- phosed	lost
Immature controls.....	13	none	1	2	2	8	13	0	0
I.....	50	T. P.	25	0	2	1	23	5	10
		T. P. ^a C. G.	2	0	10	0	11	1	
II.....	16	T. P.	4	3	0	0	6	1	2
		T. P. C. G.	0	4	3	0	6	1	
III.....	28	oil	5	8	0	0	10	3	1
		oil C. G.	0	7	7	0	11	3	

^a T. P. Testosterone propionate.
C. G. Chorionic gonadotropin.

one injections were given after sex differentiation had already occurred there were 4 males to 3 females (table I).

The administration of chorionic gonadotropin in two injections on successive days caused marked stimulation of the interstitial tissue of the gonads of all animals treated (figs. 6 and 7). There was no apparent effect on the germ cells. The chorionic gonadotropin in stimulating the interstitial tissue caused the development, or retention, of a cavity which had the appearance of a secondary ovarian cavity. The gonads of animals which were genetic males or sex-reversed males, as a result of the testosterone treatment, and which also received chorionic gonadotropin, appear to be of an intersexual type (fig. 6). In groups which received chorionic gonadotropin the sex ratios were modified from that expected with a preponderance of intersexes or females. Instead of the expected sex ratio of 1:1 in the controls and 100 percent males following testosterone treatment very few gonads were of the male type in those animals receiving chorionic gonadotropins (table I).

Whereas the larval testes stimulated with chorionic gonadotropin assume the form of gonads with much distended ovarian sacs with germ cells surrounding the cavities (fig. 1: G, H, I, J), the testes of metamorphosed males, treated with chorionic gonadotropin, resemble the testes of control males, showing the formation of seminiferous tubules and interstitial cell stimulation (figs. 8, 9, 10, 11).

Ovaries of larvae show some effects of the chorionic gonadotropin

in that the interstitial tissues are stimulated and the ovarian cavities appear to be more distended than those of controls. However, no marked differences are apparent between ovaries of metamorphosed animals treated with chorionic gonadotropins and control animals, but the small number of females prevents the drawing of any definite conclusions concerning the growth of oocytes.

The wolffian ducts of none of the groups of animals, larval or metamorphosed, show any apparent unnatural increase in size. The müllerian ducts could not be definitely identified and, as in normal animals, are undeveloped at this stage.

DISCUSSION

A single injection of 0.025 mgs. of synthetic male sex hormone (Perandren-Ciba) is sufficient to bring about reversal of sex from female to male if treatment is started before sex differentiation has occurred. Mintz (1948) has shown that 1 gamma per liter of aquarium water, administered to larvae of *Rana sylvatica*, from hatching to metamorphosis, is sufficient to bring about induction of male development. The amount of testosterone propionate injected into larvae in this experiment was greater than the amount used by Mintz. In the group of older larvae (Group II) treated with testosterone propionate no great modification of the ovaries was noted, but it is probable that if the amount of hormone had been increased and the experiment had been prolonged, reversal of females to males would have occurred. Mintz, Foote, and Witschi (1945) found that a total

of 4.0 mgs. of testosterone propionate in weekly injections brought about sex reversal in *Rana clamitans* larvae.

The chorionic gonadotropin used in this experiment ("APL"-Ayerst) contained a preponderance of lutinizing hormone. This preparation, as stated by the pharmaceutical company preparing it, is to be used therapeutically for the lutinizing effect to aid corpus luteum development. Therefore little effect on follicle stimulation could have been anticipated. Injections of this hormone into adult males of *Rana pipiens* in our laboratory caused the appearance of spermatozoa in the urine, as in cases of testing positive pregnancy urine. Treatment of larval forms with this hormone brought about no maturation of the germ cells; the entire effect of the hormone was on the interstitial tissues of the gonad. Krichesky (1934) reports that human pregnancy urine extracts administered over a period of 10 days had no effect on larvae of *Rana catesbiana*.

There have been few reports of the use of a combination of sex hormones and chorionic gonadotropins on anuran larvae. Puckett (1939) found precocious development of gonads of *Rana catesbiana* tadpoles with injections of mammalian pituitary extracts, as well as acceleration of the development of sex reversed gonads of larvae receiving both sex hormone and pituitary injections. Mintz, Foote, and Witschi (1945) using a variety of gonadotropic substances found no effect on the course of sex reversal of females to males in *Rana clamitans* larvae treated with testosterone propionate.

There are several factors involved in the variations in results reported by Puckett (1939), Mintz, Foote and Witschi (1945), and by the present investigator. Some of these are: 1.) different species of anurans were used, 2.) Puckett and Mintz, Foote, and Witschi began treatment with sex hormones and gonadotropins at a time when the sex of the larvae had already differentiated, whereas, in the present experiment, treatment with sex hormones began before sex differentiation was complete, 3.) in the experiments of Puckett and Mintz, Foote, and Witschi, treatment with the two types of hormones was simultaneous, whereas in the present experiment the gonadotropin was not administered until shortly before the animals were sacrificed, and after sex reversal had occurred, 4.) dosage of hormone was less in the present experiment, and 5.) apparently there was a greater proportion of follicle-stimulating hormone in the gonadotropic substances used in the earlier experiments, while here the gonadotropic hormone contained mostly lutinizing substances.

Engle (1939) states that the evidence from animal experiments indicates that human chorionic hormone is never a follicle stimulator and that in the male at ages below maturity, the uniform response is the hypertrophy and hyperplasia of the interstitial cells with the concomitant production of androgens. In this experiment with larval anurans there is a marked hypertrophy of interstitial cells of the gonad, but there is little evidence to indicate whether or not androgens were produced. No response of the gonoducts

was noted, even though synthetic androgen was administered. It may be that the Wolffian ducts were not mature enough to respond to androgen stimulation even though the hormone was present or perhaps the injected androgen was in insufficient amounts to bring about a response.

On the other hand, the testes, even though stimulated with chorionic gonadotropin, may produce no androgens during the larval stage. Perhaps, if the experiment could have been prolonged the testes would have produced androgens and brought about stimulation of the gonoducts. It does seem, however, that the gonads were not producing natural androgens during the larval period of development.

SUMMARY

1. A single injection of 0.025 mgs. of testosterone propionate administered to larvae before sex differentiation is complete brings about reversal of sex from female to male, after a period of two months.

2. A single injection of 0.025 mgs. of testosterone propionate administered to larvae after sex differentiation has occurred does not bring about reversal of sex, after a period of one month.

3. Two injections of 0.01 cc. (100 I.U.) of chorionic gonadotropic hormone just prior to the time of sacrifice cause stimulation of interstitial tissues of the gonads.

4. In larvae, the testes respond to chorionic gonadotropic hormone stimulation by the formation of a cavity in the gonad which is similar to the secondary gonad cavity of the ovary.

5. In metamorphosed animals the testes respond to chorionic gonadotropic hormone treatment by formation of seminiferous tubules and the hypertrophy of interstitial tissues.

6. Wolffian ducts are not stimulated by synthetic male sex hormones or by chorionic gonadotropic hormones. Mullerian ducts were undeveloped in animals observed.

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