DEVELOPMENT OF *XENOPUS* OVARIES AND TESTES GROWN TOGETHER IN ORGAN CULTURES

CHARLES L. FOOTE AND FERN PROBSTMEYER
Southern Illinois University, Carbondale

Introduction

Organ culture methods have been used to gain additional information concerning the process of sex differentiation in amphibians. The primary purpose of this study was to determine the time in larval development at which the sex inductor substances (Witschi, 1914, 1931) have their greatest influence. Comparisons are made between results presented here and those described by Chang (1953) for parabiotic experiments with *Xenopus laevis.*

This study was supported by grants from the Graduate School, Southern Illinois University, and the U. S. Public Health Service.

Materials and Methods

Ovaries and testes of *Xenopus laevis* were placed in direct contact with each other in organ cultures. Seventy-three cultures were prepared in which four pairs of explants were placed in each culture dish. Since the larvae from which gonads were taken were in different stages of development they were placed in groups designated A, B, C, D (Table 1). Developmental stages were determined from Nieuwkoop and Faber (1956).

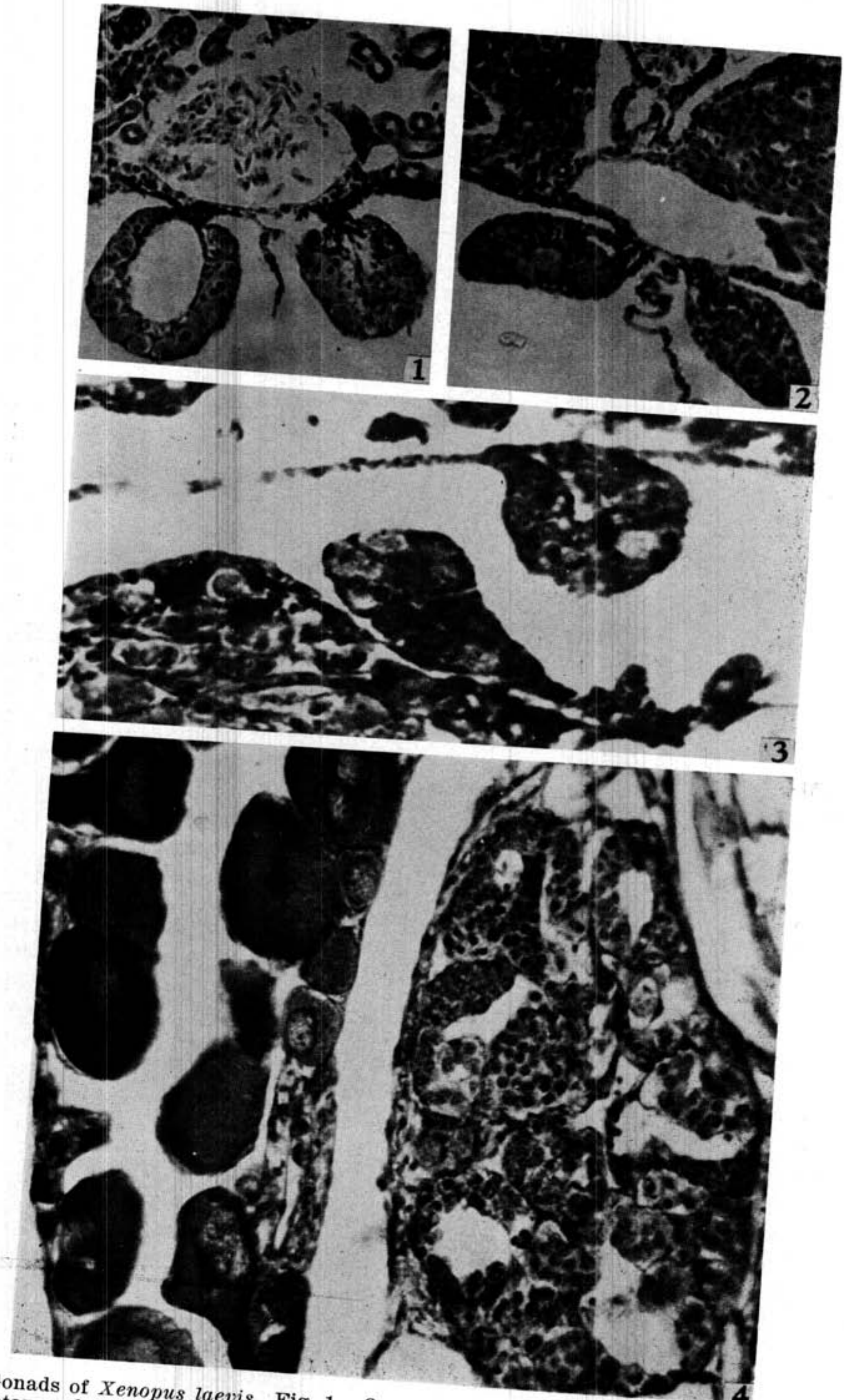
Group A consisted of gonads from tadpoles at developmental stage 53. Group B was made up of gonads from larvae of stage 53-54 combined with those from larvae of stage 56-57. Groups A and B were cultured with gonads attached to kidneys. Group C consisted of cultures in which one-third of an ovary was placed in direct contact with one entire testis, both of which had been removed from animals at the time of metamorphosis. Group D was composed of cultures of equal sized pieces of ovary and testis from two-months-old frogs.

Techniques and procedures used in preparing cultures of amphibian gonads have been described (Foote and Foote, 1958).

All cultures were continued for 30 days, with the exception of those of Group D which were maintained for 10 days. Control gonads from intact animals were fixed at the beginning and end of the culture period. *In vitro* controls were obtained by culturing individual gonads and gonad pairs of the same sex, concurrently with those in heterosexual combinations. Gonads were preserved in Zenkers's fixative, sectioned at seven microns, and strained with Harris' hematoxylin and chromotrope-2R.

Results

Controls—Gonads removed from intact larvae were larger and better differentiated than corresponding gonads maintained in culture. Uncultured gonads were more compact and germ cells were distinct (Figs. 1, 2). Control gonads which had been maintained in culture were not



Gonads of *Xenopus laevis*. Fig. 1.—Ovaries of control larva, *in vivo* just prior to metamorphosis (stage 59-60). x220. FIG. 2.—Testes of control larva, *in vivo* just prior to metamorphosis (stage 59-60). x220. FIG. 3.—One ovary from stage 53 larva, the other from stage 57, together *in vitro* for 30 days. x460. FIG. 4.—Ovary and testis, *in vitro* for 10 days, from two-months-old frogs. x130.

of uniform shape and were less well differentiated than gonads from *in vivo* controls, but germ cells could be easily recognized (Fig. 3). In Groups A and B the male-male and female-female combinations served as controls

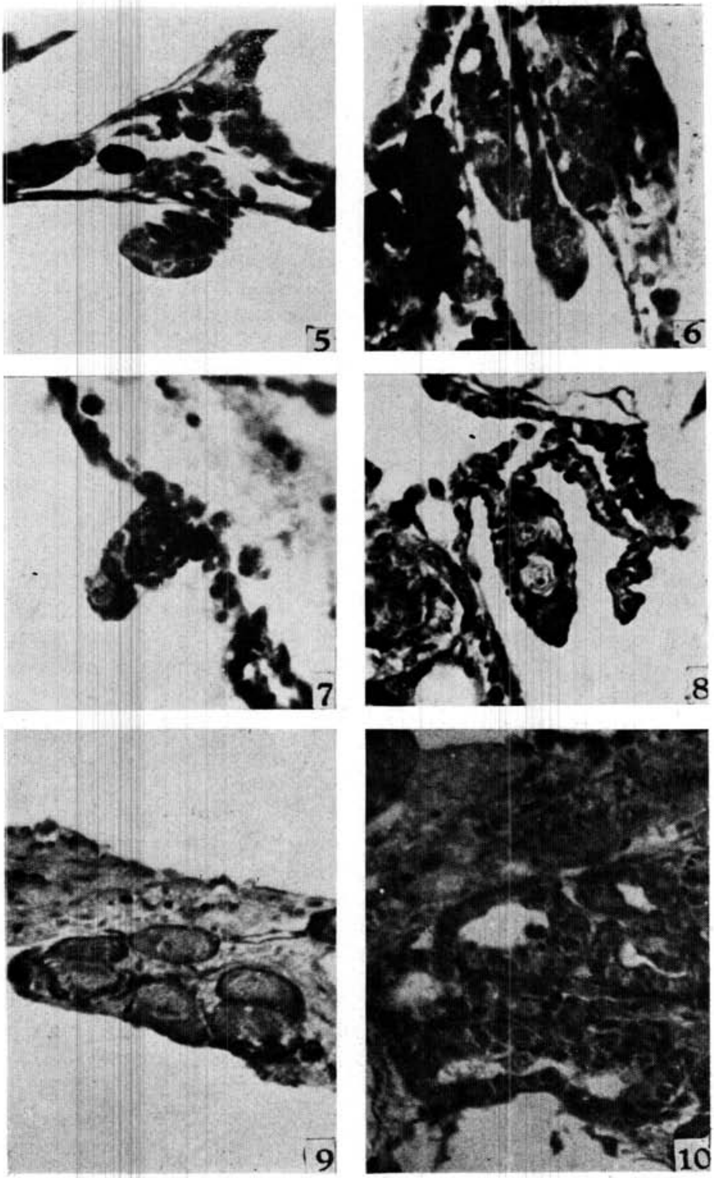
Group A—Gonads from larvae of developmental stage 53, after 30 days *in vitro,* were smaller than those from intact control animals. Although the organs were varied in shape, germ cells were distinct. There were four male-male combinations, and all these gonads were of the same size and degree of differentiation at the end of the culture period. In the three female-female combinations the ovaries were of equal size and showed the same degree of differentiation. In the nine heterosexual pairs, the testis predominated (Fig. 6). In five of these the ovaries appeared to be small and immature (Fig. 5) and in the other four pairs the testes had apparent normal structure, but gonads with which they were paired were intersexual. Since the sex of all gonads was readily apparent, some differentiation had occurred while the organs were in culture. Stage 53 is the time of early sex differentiation.

Group B—After 30 days *in vitro* these gonads were smaller than those from intact control animals, and the non-germinal cells were somewhat more dispersed. The form of the cultured gonads varied from long, slender ribbons to small cylinders, and others were of more irregular shape.

Ovaries appeared to differentiate earlier than testes. Of the 17 combinations maintained in culture there were five female-female pairs. In four of these the gonads were of the same size and showed the same degree of differentiation (Fig. 3) but in one case the younger gonads (stage 53) were less well developed. There were two male-male combinations in which the stage 53 gonads were smaller and less well differentiated than those of stage 57. In one case a testis was paired with an undifferentiated gonad. Of the nine heterosexual pairs the gonads in four were of the same degree of development. Four pairs showed inhibition of the ovarian cortex (Figs. 7, 8). One heterosexual pair was composed of a large ovary and a relatively smaller testis. No intersexes were observed. In this group where ages of larvae differed it was assumed that the larger explant came from a larva of stage 57.

Group C—Non-germinal cells intermingled at points of contact of the organs. All cells of the testes were in good condition, and in some instances the seminiferous tubules had well-developed cavities (Fig. 1). There were few ovogonia in ovarian explants and most auxocytes had degenerated (Fig.9).

Group D—At the area of contact between ovaries and testes there was an intermingling of non-germinal cells, but the germ cells remained separated. Ovaries contained auxocytes, and in some cases many ovogonia, while testes had germ cells in all stages of maturation, including mature spermatozoa. There was no change in the sex of the gonads, but those seminiferous tubules nearer the ovarian explant were more distended than in other areas (Fig. 4).



Gonads of *Xenopus laevis*. FIG. 5.—Ovaries of larva of stage 53, *in vitro* for 30 days with testes (Fig. 6). x420. FIG. 6.—Testes of larva of stage 53, *in vitro* for 30 days with ovaries (Fig. 5). x420. FIG. 7.—Ovary of larva of stage 57, *in vitro* for 30 days with testis of stage 53 (Fig. 8). x 390. FIG. 8—Testis of larva of stage 53, *in vitro* for 30 days with ovary of stage 57 (Fig. 7). x390. FIG. 9.—Ovary, *in vitro* for 30 days, from larva at metamorphosis. Paired with testis (Fig. 10). x250. FIG. 10.—Testis, *in vitro* for 30 days, from larva at metamorphosis. Paired with ovary (Fig. 9). x250.

TABLE 1.—Developmental Stages, Cultivation Time and Final Sex Distribution.

Group	Stage	Days in vitro	Explants	Final sex distribution in vitrc					Controls in vitro		Controls in vivo	
				♂-♂	♂-⚥	♂-♀	♀-♀	⚥ undiff.	♂	♀	♂	♀
A	53	30	16 pairs	4	4	5	3	0	4	3	2	3
B	53-57	30	17 pairs	2	0	9	5	1	4	3	11	12
C	At meta-morphosis	30	14 testes	0	0	14	0	0	6	6	10	4
			14 ovaries	0	0							
D	Frogs	10	26 testes									
			26 ovaries	0	0	26	0	0	5	6	7	2

DISCUSSION

Studies on frogs have shown that the inductors of sex differentiation are located in the cortex and the medulla of gonads (Witschi, 1914, 1931). Cortical substances induce ovarial differentiation, and medullary substances induce testicular differentiation.

If the cortex of a gonad of a genetic female is destroyed, the suppressed medulla is given a chance to grow and initiate testicular differentiation. In the transformation of a genetic male into a female the reverse is true, but in males the cortex usually disappears early in development. Consequently, male to female sex inversions are restricted to early embryonic or larval stages (Witschi, 1931). It has been demonstrated by parabiosis experiments that the inductor substances travel by diffusion through the tissues in frogs. They suppress differentiation of the gonads of the opposite sex which results in partial or complete sterility.

The time during embryonic development at which the progress of gonad differentiation can be influenced to the greatest degree has been investigated by Chang (1953). He reported that testes inhibit the development of ovaries of larvae of *Xenopus laevis* in heterosexual parabiotic combinations, and that the male inductor substance has its maximum potency some time after metamorphosis, but prior to sexual maturity. The female is maximally susceptible to the male inductor during the early larval period.

Results from the present experiment shows that with gonads from larvae of stage 53 (Group A) the testis predominates by depressing cortical development, and causes some ovaries to become intersexual. Heterosexual pairs where one set of gonads is from a larva of stage 53 and the other from a larva of stage 57 (Group B) give less clear results. Four, and possibly five, heterosexual pairs demonstrate that the testis is predominant since the ovarian cortex is much reduced. However, in four instances the testes and ovaries show comparable degrees of differentiation, equivalent to that of controls, and in one pair the testis is much smaller than the ovary. The male inductor substance from the older larvae depresses cortical development of the younger ovaries. However, when ovaries are from older larvae, both ovaries and testes are in comparable stages, with a single exception, at the end of the culture period. In heterosexual pairs from metamorphosing animals (Group C) ovaries develop less well than testes. In general these results substantiate the findings of Chang (1953).

The predominance of the male gonad, as indicated by results from this study, suggests that the substance depressing ovarian development is not a sex hormone, since it has been shown that certain estrogens and androgens bring about reversal of males to females (Witschi and Allison, 1950; Chang and Witschi, 1955, 1956; Gallien, 1956).

Several factors must be considered in comparing results from the present *in vitro* studies and those from *in vivo* experiments. Gonads maintained in culture do not develop and differentiate as rapidly as do those of control animals *in vivo*. Consequently, at the end of the culture period, organs may not be in the same stage as those from intact animals of comparable ages. Another factor is the transmission of the inductor substances from one pair of gonads to the other in parabiosis and in culture, by diffusion through the tissues or the medium surrounding the gonads. The distance between gonads may be of little significance since no marked differences are observed between heterosexual pairs that are in direct contact and those some distance apart. Thirdly, gonads in culture apparently became modified biochemically, as has been indicated when gonad explants are transplanted into intact larvae after having been in culture for from 30 to 42 days (Foote, Mathews, and Foote, 1959).

Summary

1. Ovaries and testes of tadpoles and frogs of *Xenopus laevis* of varying ages were grown together in organ cultures on a medium of chick embryo extract and chicken plasma.

2. Heterosexual combinations of gonads made at time of early sex differentiation demonstrate that the testis is the predominant organ, depressing ovarial activity and influencing the formation of intersexes

3. Gonads grown in combination from larvae of different ages show that testes depress ovarian development in 50% of the heterosexual combinations.

4. In ovary-testis pairs from larvae during metamorphosis the testes are better differentiated.

5. No marked changes are observed in the ovaries and testes grown together if they are from frogs two months old.

6. Sex inductor substances are present in gonads during early sex differentiation and the inductor from the medullary area of the gonad is predominant until soon after metamorphosis.

Literature Cited

Chang, Chih Ye. 1953. Parabiosis and gonad transplantation in *Xenopus laevis* Daudin. Jour. Exper. Zool., 123: 1-28.

CHANG, CHIH YE, and EMIL WITSCHI. 1955. Breeding of sex-reversed males of *Xenopus laevis* Daudin. Proc. Soc. Exper. Biol. Med., 89:150-152.

1956. Genic control and hormonal reversal of sex differentiation in *Xenopus*. Proc. Soc. Exper. Biol. Med., 93: 140-144.

FOOTE, C. L., and F. M. FOOTE. 1958. *In vitro* cultivation of gonads of larval anurans. Anat. Rec., 130:553-565.

FOOTE, C. L., W. B. MATHEWS, JR., and F. M. FOOTE. 1959. Gonads maintained *in vitro* and grafted into castrate and intact larvae of *Rana catesbeiana*. Anat. Rec., 134:564.

GALLIEN, L. 1956. Inversion expèrimentale du sexe chez un anoure infèrieur *Xenopus laevis* Daudin. Analyse des consèquences gènètiques. Bull. Biol. de France et Belg., 90:163-183.

NIEUWKOOP, P. D., and J. FABER (eds.). 1956. Normal table of *Xenopus laevis* (Daudin). Amsterdam, North-Holland Publ. Co., 243 pp.

WITSCHI, EMIL. 1914. Studien über die Geschlechtsbestimmung bei Fröschen. Arch. Mikr. Anat., 86:1-50.

1931. Studies on sex differentiation and sex determination in amphibians. V. Range of the cortex-medulla antagonism in parabiotic twins of Ranidae and Hylidae. Jour. Exper. Zool., 58: 113-145.

WITSCHI, EMIL, and J. ALLISON. 1950. Responses of *Xenopus* and *Alytes* to the administration of some steroid hormones. Anat. Rec., 108:589-590.

Manuscript received May 18, 1959.