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**Sex Reversal in *Amblystoma*. VI. Interactions of Ovary and Testis in Arkansas Strain of *Amblystoma Punctatum*.\***

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In experiments in which one gonadic preprimordium of an embryo was replaced by that of another, it was found that in the local (Buffalo, N. Y.) strain of *A. punctatum* an ovary developing in the presence of a testis was commonly modified under its influence and eventually reduced to the so-called freemartin condition.<sup>1</sup> In such an ovary the formation of the ovarial sacs (central cavity) is suppressed and the growth of the cortex is inhibited; the gonad is thus eventually reduced to a rudimentary or atrophic structure in which only a few oögonia remain. A comparable modification of the ovaries has been induced in females of this strain joined in parabiosis with males.<sup>2</sup>

In the experiment here reported, transplants of the gonadic preprimordium were made in embryos of this same species but of a local race or strain secured from Arkansas. In this strain the ovaries of ungrafted females were found to develop normally, while the gonads of the males commonly exhibited a more pronounced bisexual or hermaphroditic condition than is usually encountered in males of this species. In many cases the gonad had the structure of a testis in its caudal portion, with its cephalic end tending to resemble an ovary (Figs. 1 and 2), but in even more cases the bisexuality consisted rather in a pronounced development of the covering germinal epithelium to give a prominent 'male cortex' over a large part of the length of the gonad (Fig. 3). In males under 4 months of age this cortex was sometimes found to be hypertrophied, with many of its germ cells in the spireme stages characteristic of early oöcytes; in such gonads the medullary or testicular portion was either poorly developed or possessed cavities of greater or less extent (comparable to those of ovarial sacs) in which numerous spermatogonia lay free, often in some phase of degeneration (Fig. 4). With further extension of these cavities and degeneration of the spermatogonia con-

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<sup>1</sup> Humphrey, R. R., *J. Exp. Zool.*, 1929, **53**, 171.

<sup>2</sup> Humphrey, R. R., *Proc. Soc. Exp. Biol. and Med.*, 1932, **29**, 713.

tained in them, the gonad became transformed into an ovary (Fig. 5), which, after further development of its cortex and the final disappearance of the spermatogonia, became indistinguishable from the ovaries of zygotic females. It was concluded that in all about 40% of the zygotic males underwent transformation into females.

Despite the bisexual or hermaphroditic character of the testes in this strain and their tendency to transform into ovaries as above described, it was found that in the grafted animals an ovary in the presence of a testis was inhibited and modified thereby in 25 out of 59 possible cases (42%). Of the 16 animals of this group killed at 2 to 5 months of age, only 8 (50%) showed enlarged oöcytes (auxocytes) in the modified ovary as in Figs. 6 and 7. These cells probably would have disappeared eventually, since many could be found undergoing cytolysis. In 6 of these older animals (38%) no large oöcytes were to be found in the modified ovary, while in 2 (12%) only oögonia remained (Figs. 8 and 9).

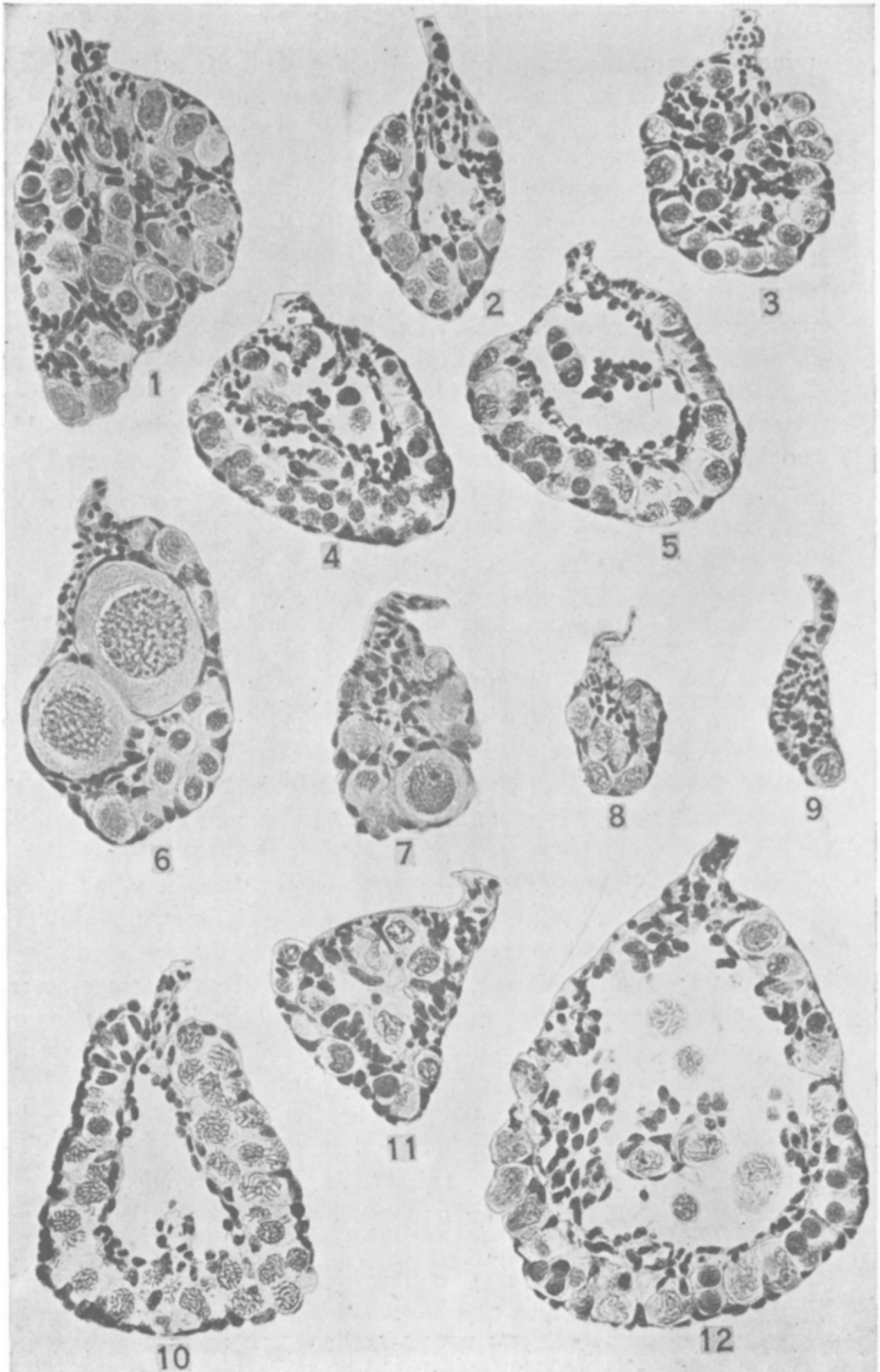
In 34 out of 59 possible cases (58%) the ovary developed in the presence of a testis was either normal or but little modified (Fig. 10) while the associated testis (either that of graft or host) showed more pronounced modification (Fig. 11) or even a more or less complete transformation into an ovary (Fig. 12). That such reversal was in part dependent upon the influence of the associated ovary is indicated by the fact that it occurred more frequently here than in ungrafted animals (58% of all possible cases as compared with 40% in ungrafted animals).

It is possible that the 'spontaneous' reversal of males noted above may have been due to the low temperature at which the animals were reared, since Witschi states that development of the medullary portion of the gonad in *Amblystoma* is delayed by low temperature, which thus favors the growth of the cortex and the differentiation of the gonad as an ovary.<sup>3</sup> In any event, it would appear that the zygotic males whose gonads transformed into ovaries did not later revert to males in the manner described by Witschi for the grass frog, since 70% of the animals over 4 months of age proved to be females, whereas among the animals of 2 to 3 months, approximately 50% could be classified as males either normal or undergoing reversal.

It must be emphasized that no gonad in animals of this strain was found to develop first as a typical ovary, with subsequent transform-

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<sup>3</sup> Witschi, E., *Sex and Internal Secretions*, 1932, Chapter V, Williams and Wilkins, Baltimore.



All figures are photographs of transections of gonads,  $\times 170$ .

- Fig. 1. Caudal end of bisexual gonad, male of 110 days.  
 Fig. 2. Cephalic end of gonad shown in Fig. 1.  
 Fig. 3. Testis with prominent cortex, male of 80 days.  
 Fig. 4. Early cavity formation with degeneration of spermatogonia, male of 80 days.  
 Fig. 5. Same gonad as in Fig. 4, but showing a region with fewer spermatogonia and cortex more advanced.  
 Fig. 6. Modified ovary, in male host of 140 days.  
 Fig. 7. Modified ovary of female carrying a testis graft. 109 days.  
 Fig. 8. Modified ovary of female carrying a testis graft. 148 days.  
 Fig. 9. Graft ovary, freemartin type, from male host of 148 days.  
 Fig. 10. Graft ovary of normal structure in male host of 80 days.  
 Fig. 11. Modified testis of host carrying the graft ovary shown in Fig. 10.  
 Fig. 12. Testis in reversal, from a host of 110 days, carrying a graft ovary.  
 Note degenerating spermatogonia in central cavity.

ation to a testis, as is seen in the so-called undifferentiated races of frogs. After the onset of sex-differentiation, the gonads of zygotic males were generally found to be of testicular structure in a large part of their extent. When the medullary (testicular) component of such a bisexual gonad was able to inhibit the hypertrophy of the ovarian component (either cephalic ovarian portion, or continuous 'male cortex') and so escape reversal, it was commonly able to inhibit and modify the growth of an ovary developing with it in the same animal or reduce that ovary to a freemartin condition. It would therefore seem probable that the testicular substance ('medullarian') antagonistic to cortical (ovarian) growth and differentiation was released into the blood stream as well as transferred by diffusion into the adjacent cortex of the bisexual gonad.

The reduction of the ovary to a freemartin type in a considerable proportion of cases in the present experiment is in contrast with the results obtained by Witschi in an Ozark Mountain (Arkansas) strain of *A. punctatum*.<sup>4</sup> Witschi states that "in heterosexual parabiotic twins of this race the gonads of the female mate develop almost normally (no freemartin effect in this case)". Whether this is due to the decreased intensity of the interaction of the gonads in parabiotic twins as compared with grafted animals, or whether the animals used by Witschi are actually of a different local race than that studied by the writer can not be stated. My findings, however, support Witschi's conclusion that "sex antagonism is low in forms that constitutionally are inclined toward hermaphroditism," since they indicate the action of the testis upon the ovary to be either late in onset or of relatively low intensity.

<sup>4</sup> Witschi, E., *Anat. Rec.*, 1932, **54**, 76.