67. *Influence of Follicular Hormone Upon the Sex Differentiation in Hynobius retardatus.*

By Kin-itiro HANAOKA.

Zoological Institute, Faculty of Science, Hokkaido Imperial University, Sapporo.

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In 1936 Padoa¹⁾ reported that the water solution of female sex hormone, in which the tadpoles of *Rana esculenta* were raised, induced the differentiation of male gonads in the majority of experimental animals. Witschi and Crown (1938)²⁾ repeated the same experiment with *R. pipens*, but failed to obtain the masculinizing effect of estrogen. Burns (1938,³⁾ 1939),⁴⁾ examined the influence of sex hormone on *Amblystoma punctatum* by the method of injection. He found that the injection of crystalline follicular hormone caused transformation of testes into ovaries. The administration of the male hormone, on the other hand, induced the differentiation of hermaphroditic gonads in the genetical females.

The experiment described in the present paper deals with the injection of follicular hormone in a Japanese salamander, *Hynobius retardatus*. This work has been carried out under the guidance of Professor Uchida and by a grant from the Tōsyōgū Tercentenary Foundation.

Material and method. *H. retardatus* is a semidifferentiated race in regard to sex and in the early larval stages the testis takes the structure of ovary. The male differentiation begins with the onset of metamorphosis at two or three months after fertilization. At the end of metamorphosis the sex ratio is nearly 50 to 50 (Hanaoka, 1934).⁵⁾ At the beginning of the experiment, the larvae were 21 or 22 mm in length when their gonads had just appeared. The hormone used was pelanin (Tōkyō Zōkiyaku-Kenkyusyo). It contains 500 m. u. of follicular hormone in every cubic centimeter. The larvae received the injection of about 5 m. u. of follicular hormone at 5-7 day intervals over the period of 2-3 months.

Results.

I. Before or during metamorphosis. 55 animals received the injection and 26 of them were preserved before or during metamorphosis at the age of about two months. The remaining 29 were sacrificed one or two months after metamorphosis. At autopsy it was found that the gonads were differently modified according to the ages of the animals.

1) Monit. Zool. Ital., vol. 47, pp. 285-289.

2) Anat. Rec., vol. 70 (suppl. no. 1), p. 121.

3) Anat. Rec., vol. 71, pp. 448-461.

4) Anat. Rec., vol. 73, pp. 73-93.

5) Jour. Fac. Sci., Hokkaido Imp. Univ., vol. 3, no. 4, pp. 247-253.

In 26 animals preserved before or during metamorphosis the testes and ovaries were similar in shape and looked like slender threads hanging on the mid-ventral line of the kidney. Their histologic structure was similar in every respect to that of gonads of normal animals of the similar size. The testis was in the early stage of rete cord formation and the ovary had a distinct ovarian cavity and cortical germ layer; neither modification of testicular differentiation nor acceleration of the ovarian development was detected.

II. One month after metamorphosis. The gonads of 18 animals preserved one month after metamorphosis were in different conditions. They were more or less shortened, and most of them had white nodular protruberance on the surface. 13 of the 18 animals were genetical females with the normal ovaries having a distinct ovarian cavity and a cortical germ layer. But germ cells were more differentiated than in the normal gonads and each had a large vesicular nucleus and dense protoplasmic substance (Fig. 1). Out of 5 genetical males two had testes normally differentiated for their ages. In the other three the rete cord was nearly normal and most of the germ cells were normal

Fig. 1. Section through an ovary of a female one month after metamorphosis. $\times 150$

Fig. 2. Mosaic gonad of a male one month after metamorphosis. $\times 150$

in structure but, intermingling with the normal germ cells, one could find many large germ cells not different in structure from those found in the ovaries of the experimental female. Thus the gonads on the whole were the mosaic structures of male and female characteristics (Fig. 2).

III. Two months after metamorphosis. In animals sacrificed two months after metamorphosis the effect of the hormone injection was more conspicuous. In the females differentiation of oocytes proceeded one or two years ahead of the normal ones, the ovarian cavity having become almost occluded by enlarged oocytes (Fig. 3). In mosaic gonads of the males the egg cells were larger

Fig. 3. Section through an ovary of a female two months after metamorphosis. $\times 100$

Fig. 4. Mosaic gonad of a male one month after metamorphosis. $\times 100$

and more numerous. Fig. 4 represents a section of a mosaic gonad showing the differentiation of rete cord on one end and a large egg cell on the other. No sign of cavity formation was observed.

In closing this section the essential data may be tabulated as follows.

TABLE 1.

Distribution of sex in H. retardatus injected with pelanin.

	♂	♀	♀	TOTAL
Before or during metamorphosis	15		11	26
One month after metamorphosis	2	3	11	18
Two months after metamorphosis		4	7	11

Discussion.

The result of the present experiment is diametrically opposite to those of Padoa. The masculinizing effect of the female sex hormone reported in *R. esculenta* was not at all observed in *H. retardatus*. The two experiments, however, are different in the method of administration of hormone. As the experiment similar to Padoa's is being carried on by the present writer on *H. retardatus*, it would be better not to go into the discussion at present. The result obtained with *H. retardatus* differs also in some important points from Burns' with *A. punctatum*. Burns states that transformation of testes into ovaries is actually induced by the injection of female sex hormone and the ovarian cavity is formed in the genetical male gonads, but in the female no acceleration of ovarian differentiation is observed. In *H. retardatus*, on the contrary, the acceleration of ovarian differentiation was observed in the female and in the male no reversal of testes into ovaries occurred. It may, therefore, be said that in this form female hormone exerts its influence only on the germ cell differentiation and not on the differentiation of the gonad. The medulla of the male gonad always developed as in the normal male and a certain number of germ cells which differentiated toward the female direction are found intermingling with normal male germ cells. In parabiotic twins of *A. punctatum* Burns (1935)¹⁾ reported that the genetical male affected by a female partner differentiated into a female. The two experiments of Burns show, therefore, that the postpuberal hormone can act similarly as inductive influence, in the sense of Witschi. Witschi, (1937)²⁾ contended, how-

1) Anat. Rec., vol. 63, pp. 101-129.

2) J. Exp. Zool., 75, pp. 317-359.

ever, on the basis of his experiments on parabiosis in various forms of urodeles, that inductive action of medulla as well as of cortex is of different nature from the action of postpuberal hormone, the one acting on the differentiation of structure of the gonad, and the other on the differentiation of germ cells. The present experiment shows that the medullary induction of genetical male is effective independently of the action of female postpuberal hormone, and the gonad is therefore always solid, while the germ cells therein contained turn into eggs.