A SEMIDIFFERENTIATED RACE OF URODELANS WITH REGARD TO ITS GONADIC DEVELOPMENT[1)]

BY

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(*With Two Plates and Three Text-figures*)

Since WITSCHI's work (1914) on the hermaphroditic tendency of young individuals of frogs, *Rana temporaria* and *R. esculenta*, it has been clear that in the species of some European regions there are races undifferentiated in regard to sexuality. SWINGLE (1925) reported another case of an undifferentiated race of *Rana catesbiana*, and pointed out that the phenomenon is not included in the category of sex reversal but is due to a modified case of normal development in which a hermaphroditic tendency is seen. In 1929 WITSCHI distinguished in *Rana temporaria* three local races according to the types of development of the male germ gland, *i*, *e*, differentiated, undifferentiated and semidifferentiated ones, the last being the intermediate form of the first two. Later, in 1934, the same author reported experiments of parabiotic twins in two different local races of spotted salamander *Amblystoma maculatum*, desrcibing their gonadic development. Incidental to experimental studies of sexuality in the salamander *Hynobius retardatus*, the present writer studied the normal development of gonad in this form and happened to find another semidifferentiated type of sex differentiation which will be briefly described below. The writer's appreciation and

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indebtedness should be expressed to Prof. Tohru UCHIDA for his helpful criticism and encouragement.

Material and Methods

The breeding habits of *Hynobius retardatus* observed by the writer generally agree with the findings of SASAKI (1924). Fresh eggs laid in open fields were obtained from the environs of Sapporo in the early spring of 1932. At the time of collection they were all in one- or two-cell stages of cleavage. They were kept in the laboratory in running tapwater, of which the temperature was within the range of 12–13C°. After the hatching out of the larvae the temperature was gradually raised to room temperature and they were fed with fresh beef. BOUIN's solution was used for fixing, the sections were cut in 10μ and stained with EHRLICH's Haematoxylin and Eosin.

Formation of primordial germ gland

In larvae 30 days old, 19–20 mm in length and with fore limb-buds, the rudimentary gonads containing a few germ cells are found in the spaces between the dorsal root of the mesentery and the Wolffian ducts. They closely resemble the archenteron cells, heavily laden with yolk granules. In the anterior region the germ cells are divided by the mesentery and arranged in two longitudinal rows, but in the posterior region they are fused to a median row embedded in the mesentery, and, further backwards, are so sparsely distributed in the dorsal wall of the intestine that one can hardly distinguish the germ cells from the endoderm cells.

The first sign of differentiation of the germ gland was found in a larva 21 mm in total length (Text-fig. 1). In this individual the germ cells containing large yolk granules are projected a little into the coelomic cavity. The germ cells are at this stage separated into two rows throughout the whole length. Though yolk absorption is completed in the other regions of the body, large yolk granules

always remain in the germ cells, the mesonephric blastema, the walls of Wolffian ducts and the intestine.

In larvae 55 days old, about 28 mm in length, with fore-limbs of 2.5 mm and hind-limb-buds, the cells forming the germ gland are

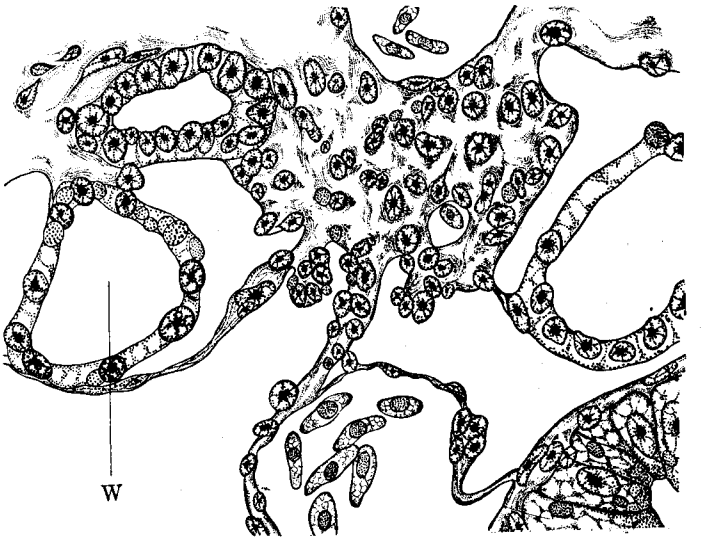

Fig. 1. Section of germ gland of larva 35 days old, 21 mm in length. W Wolffian duct. ×400.

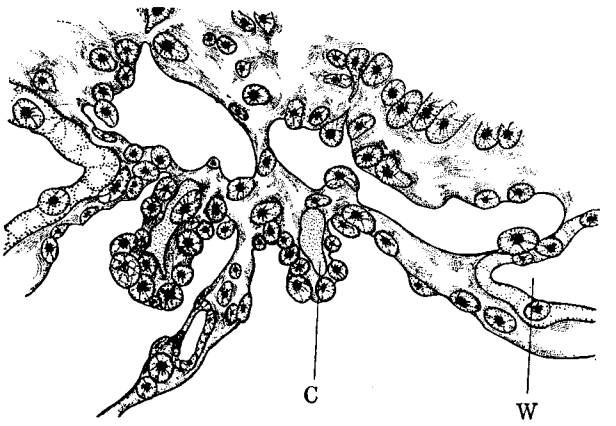

Fig. 2. Section of germ gland of larva 55 days old, 28 mm in length. C primary gonad cavity, W Wolffian duct. ×400.

arranged on the periphery with a primary cavity in the center, which is packed with some protoplasmic substance (Text-fig. 2). The nuclei of the germ cells now increased in size, are sometimes characterized by the deposition of pigment in them.

In larvae of 28–30 mm long, the fat-bodies make their appearance (Text-fig. 3). They are at first mere thickenings of peritoneum along the median root of the germ glands, but they increase in size rapidly by the successive immigration of the mesonephric blastema. After the vacuolization of the cells sets in, the fat-bodies become very large, surpassing the germ gland in volume.

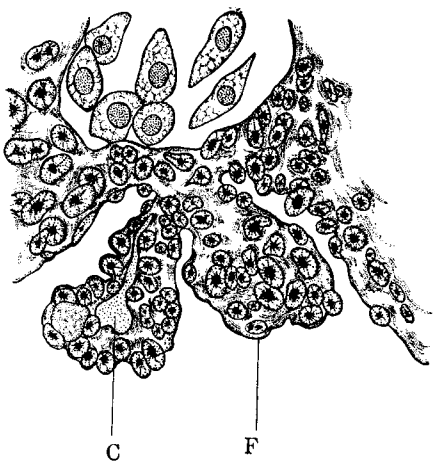

Fig. 3. Section of germ gland of larva 60 days old, 30 mm in length. C primary gonad cavity. F fat-body. ×400.

In the meantime, the immigration of the mesonephric blastema occurs in the germ gland, and in larvae 70 days old and 35–40 mm long the primary gonad cavity is fully packed with mesonephric blastema, which form the future rete cords (Fig. 1, Pl. XV). In this stage the germ glands are quite neutral, having the germinal epithlium on the peripheral and the rete cord in the central position. The germ gland is wrapped by the peritoneal epithlium and contains, within the epithlium, germ cells which are arranged in a single row and surrounded by the follicle cells.

Sex differentiation

Development of ovary. The differentiation of the ovary in the neutral germ gland is normal, as shown below. The rapid immigration of the mesonephric blastema into the germ gland ceases and the

rete cells once filling the gonad cavity become gradually arranged in the cortex region with an open cavity in the center, as shown in a tadpole 78 days old, 42–50 mm long (Fig. 2, Pl. XV). Along with the growth of larvae, the central cavity widens, becomes lined with rete cells, and eventually forms the ovarian sac. There remain, however, in the anterior and posterior ends of the gonads two small parts without a cavity. They are the epigonad and progonad, each of which always remains compact and sterile in the animal. Further description will deal exclusively with the fertile central section of the gonad.

After this stage, the germ cells increase in number and become easily distinguishable from the follicle cells (Fig. 3, Pl XV). The gonad maintains this condition during metamorphosis. In Fig. 4 (Pl. XV) photographed from a section of ovary of an animal three months old, a week after metamorphosis, one can observe the following. Only a single germ cell is enclosed in the follicle cells near the hilium region, while in the distal many germ cells are present, showing multiplication by division, two or more sister cells being enclosed in a common follicle. The germ cells thus proliferated are arranged in two or three rows around the rete cells lining the ovarian sac. The peritoneum covers the whole germ gland. In the mesovarium only a rudiment of the rete cells is found remaining. The gonads have acquired the characteristic structure of the ovaries.

Differentiation of testis. The differentiation of male gonads is characteristic in this species. At the beginning of metamorphosis the male gonads are not different from the ovary as regards the position of germ cells and the characters of medulla as shown in Fig. 5 (Pl. XV). Between Fig. 5, made from a section of a right male gonad just before metamorphosis, and Fig. 4, made from a female, we can find scarcely any essential distinction of morphological character. In Fig. 6 (Pl. XVI), however, which was photographed from a section of the left gonad of the same animal as in Fig. 5, the matter is quite different. In this gonad one finds the violent immigra-

tion of numerous mesonephric blatsema narrowed secondary cavity due to ingrowth of the rete and the detached germ cells from the peritoneum which remove toward the rete. As the rete grows, the ovarian cavity become further narrowed (Fig. 7, Pl. XVI); in a metamorphosed animal a quite small cavity is found remaining in the distal part of the gonad (Fig. 8, Pl. XVI). In Fig. 9 (Pl. XVI), made from a section of a gonad of an animal already metamorphosed, the ovarian cavity is now completely packed with rete cells having germ cells intermingled among them. In the center of the gonad, however, one can find a comparatively hyaline region which is probably the remnant of the ovarian cavity. The male differentiation of the gonad occurs at different stages according to individuals: in some specimens the development is switched into a male direction about a week before the metamorphosis, while in some the change takes place two or three weeks later.

Development of Müllerian duct

A short time before the metamorphosis, the primordia of the Müllerian duct appear along the lateral edges of the mesonephros (Fig. 10, Pl. XVI). They are at first represented by a mere thickening of the peritoneal epithelium. In animals which have completed the metamorphosis, the peritoneal epithelium is folded, and the cells found inside bearing dark nuclei, constitute the anlage of the Müllerian ducts (Fig. 11, Pl. XVI). The secondary sex characters are not distinct until at least two or three weeks after metamorphosis.

Discussion

According to WITSCHI, the differentiation of the male sex in the undifferentiated race of *Rana temporaria* begins a long time after the metamorphosis. Before the male differentiation begins, the gonad is entirely of ovarian character; even yolk formations are observed in the germ cells of future male animals, which, however, degenerate with the onset of male transformation. The case of

Hynobius retardatus here described is not so remarkable as that seen in *Rana temporaria,* but seems to be more marked than in *Amblystoma maculatum,* which has a small ovarian cavity in the male. Therefore *Hynobius retardatus* seems to be classified after the definition of WITSCHI into the semidifferentiated race in regard to its gonadic development. The process of gonadic development may be summarized as follows:

1) In the primordial germ gland a primary gonad cavity is formed in a central position which immediately afterwards becomes solid with compact rete cells.

2) The development of the ovary is normal.

3) The development of the male gonads proceeds at first in exactly the same way as the female, having an ovarian cavity and a well developed cortex. But during the metamorphosis the ovarian cavity becomes gradually occluded by the rete cells, and germ cells migrate from the cortex toward the medulla.

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Plate XV

Explanation of Plate XV

Fig. 1. Section of germ gland of larva 70 days old, 40 mm in length. ×320

Fig. 2. Section of germ gland of larva 78 days old, 45 mm in length. ×320

Fig. 3. Section of germ gland of larva 90 days old, 55 mm in length. ×320

Fig. 4. Section of ovary of metamorphosed animal, 55 mm in length. ×320

Fig. 5. Section of germ gland of male just before metamorphosis. Right side. ×320

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

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Plate XVI

Explanation of Plate XVI

Fig. 6. Section of germ gland of the same animal in Fig. 5. Left side. ×320

Fig. 7, 8. Advanced forms of male gonads. ×320

Fig. 9. Section of male germ gland of metamorphosed animal. ×320

Fig. 10. Anlage of Müllerian duct of animal before metamorphosis. ×320

Fig. 11. Anlage of Müllerian duct of metamorphosed animal. ×320

Fig. 6.

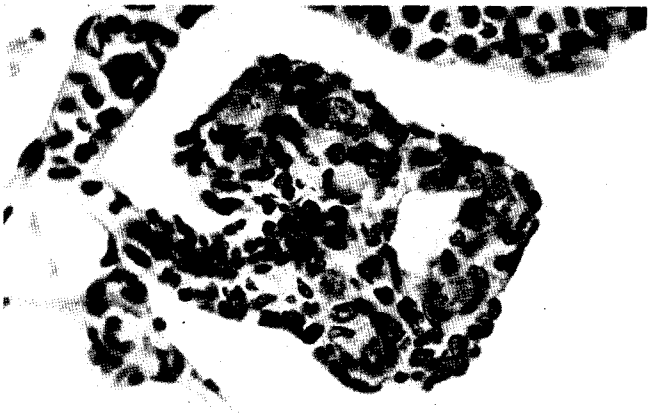

Fig. 8.

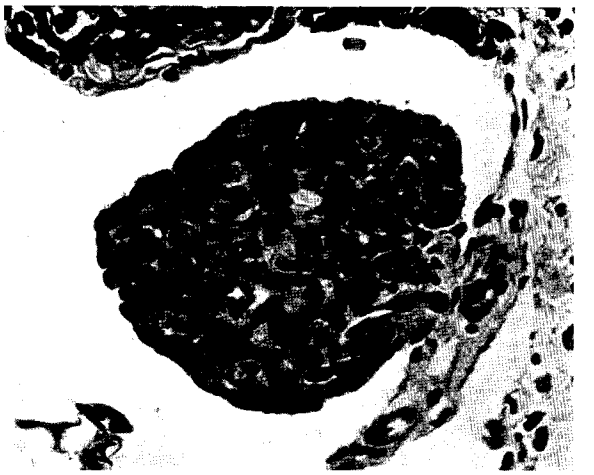

Fig. 9.

Fig. 7.

Fig. 11.

Fig. 10.

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