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# Some Observations on the Masculinizing Effect of Estradiol upon the Larval Gonads of *Rana temporaria*<sup>1)</sup>

By

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(With 13 Text-figures)

Since Padoa ('36) reported paradoxical masculinization of the larvae of *Rana esculenta* by the use of Cristallovor, it has been well established by many investigators that in sex differentiation of some anurans administration of the natural estrogen in small doses induces feminization, while in large doses it induces masculinization (Padoa & Baldasseroni, '38; Foote, '38; Gallien, '40; Padoa, '42; Witschi, '53). The present paper reports some histological observations on the effect of estradiol in high concentration upon the development of the larval gonads in a sexually semi-differentiated race of *Rana temporaria*.

It is the writer's great pleasure to be permitted to dedicate this paper to Professor Tohru Uchida, under whose direction this work has been carried out, in commemoration of his sixtieth birthday. The writer is greatly indebted to Mr. Kazuya Mikamo for his kind advice and to Mr. Junzō Ise of the Teikoku Hormone Mfg. Co., Tokyo, for supplying the crystalline sex hormones.

## Material and method

Tadpoles from a single natural spawn of *Rana temporaria* collected in Sapporo were divided, immediately after hatching, into a control group of 110 animals and four experimental groups totaling 200. They were reared at room temperature ranging from 14° to 21°C. All experimental animals were placed in a water solution of 1 mg/l of crystalline estradiol (Teikoku Hormone Mfg. Co.), and the solution was renewed every second day. The time and the duration of the treatment in each experimental group are shown in Table 1. Some of the animals were fixed at 25 and 35 days after hatching and the rest at the end of metamorphosis. Sections were cut 10  $\mu$  in thickness and stained with Delafield's hematoxylin and eosin.

## Results

Sex distribution in control and experimental groups is summarized in Table 1. Observations on the control animals proved that the frog used in this study belongs to a sexually semi-differentiated race (Fig. 1, genetical female; Fig. 2,

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Table 1. Sex distribution, time of treatment, and mortality in the metamorphosing animals of control and experimental groups

Groups	Types of the gonads*						Total	Mortality (%)	Age after hatching (days)	
	♀ PA	♀ (PA)	♀ P	♀ (P)	♂	♂			At the beginning of treatment	At the stoppage of treatment**
Control	39		12	6	21	3	81	2.0		
	39		42							
Experimental										
I.					24	7	31	17.9	5	51
II.	13	6	7	12	1		39	9.3	5	24
III.		27		4	12	5	48	1.9	25	51
IV.		12		11	4		27	3.3	35	51

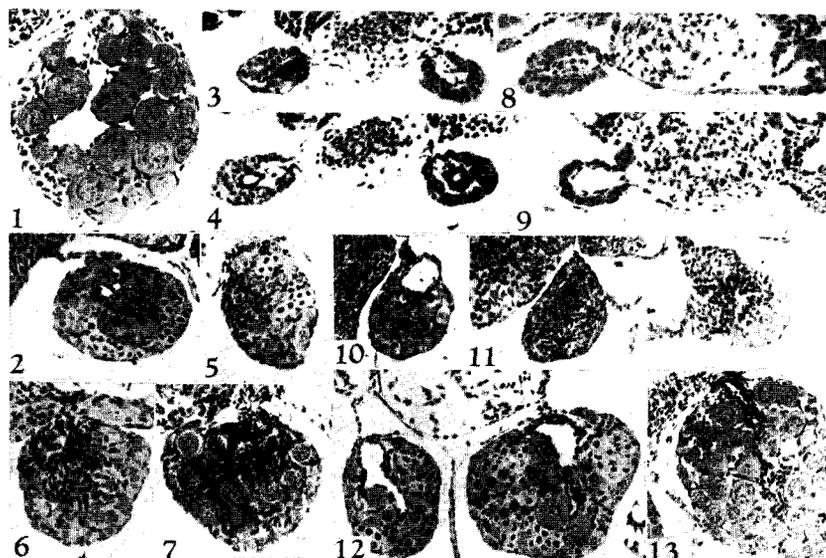
\* The signs indicating the types of the gonads. ♀ PA, normal ovaries with auxocytes; ♀ (PA), modified ovaries with auxocytes in which wall of the ovarian sacs was more or less thickened; ♀ P, normal ovaries with no auxocyte; ♀ (P), modified ovaries with no auxocyte; ♂, intersexual gonads, which were predominantly testes in Group I; ♂, differentiated testes.

\*\* It took about 45 to 50 days from hatching to completion of metamorphosis.

genetical male). As a rule, in the ovarian gonads of genetical males the germ cells did not develop into the auxocyte stage at the end of metamorphosis. At 25 days after hatching, the control animals had gonads of ovarian type, with some premeiotic oocytes in the cortex, showing the process of formation of the ovarian sacs (Figs. 3, 4).

The masculinizing action of estradiol was more or less noticeable in all treated groups and was nearly complete in the metamorphosing animals of Group I (Figs. 5, 6). The effect was first observed in the suppression of oogenesis. In the treated animals of 25 days after hatching, the cortical portion of the gonads was formed of some gonial germ cells only (Fig. 8). In Groups III and IV, the premeiotic oocytes were generally less in number at the end of the treatment than those in the controls. The suppression of oogenesis, however, was not always perfect, since even in Group I the treatment could not bring about complete absence of the premeiotic oocytes in the testes (Fig. 5). In Group II, differentiation of ovarian elements in both sexes was gradually restored after cessation of the treatment, though it was distinctly retarded in comparison with the normals (Fig. 12). Furthermore, under the hormonal influence, the development of the auxocytes was not restrained in Groups III and IV, but they were smaller in number than those in the controls. Degeneration of them was inconspicuous even when they were surrounded with medullary tissue. At 25 days after hatching, the development of the medullary tissue in the affected gonads appeared to be somewhat suppressed especially in the caudal part. Retardation of male differentiation in Group II may be ascribed to this effect of inhibition of medullary tissue. On

the other hand, in the metamorphosing animals of Groups III and IV, all the ovaries were provided with thickened wall of the ovarian sacs or with compact medullary tissue which filled up the ovarian sacs and occasionally enveloped the auxocytes in the periphery (Figs. 7, 13).



Figs. 1-13. All figures showing cross sections of the gonads on 25th day after hatching (3-4, 8-9) and at the end of metamorphosis (1-2, 5-7, 10-13). 1-4; Controls. 5-6, 8-11; Group I. 12; Group II. 7; Group III. 13; Group IV. In 8 and 9, the cavities in the center of the gonads and the opening into the caval vein at the hilum are revealed. 11 showing the distended epigonad (left), in which compact mass of blood cells is present, and the caudal end of the testis (right). Other detailed explanations in text. Magnification :  $\times 90$ .

Some histological features seen in the gonads of treated larvae of 25 days after hatching (Groups I and II) call for special attention. In 14 animals out of 22, the center of the gonads was cephalo-caudally occupied by a row of separate cavities which were segmentally demarcated by rete cords, and generally each cavity was connected with caval vein by an opening (Figs. 8, 9). Under normal conditions there was observed only loose mesenchyme tissue of the primary gonadal cavity with blood capillaries (Figs. 3, 4). In a few cases in Group I a similar abnormal cavity was found under the proximal peritoneal covering of the testes (Fig. 10). In 3 animals, such a cavity was not noticed in the corresponding parts, though the mesenchyme appeared to be abortive. In the gonads of the other 5 animals, the part between the segmental rete cords was changed into a

small crest of somatic cells with a few germ cells in process of being eliminated. The writer is inclined to consider that the latter two cases express the two extremes in the suppressive effects on the mesenchyme. Furthermore, it must be noted that the epigonads of some metamorphosing animals, chiefly in Group I, were highly distended, the central space being packed with blood cells (Fig. 11).

### Discussion

It is evident that sex differentiation of sexually semi-differentiated larvae of *Rana temporaria* tended toward masculinization as a result of administration of 1 mg/l of estradiol. Padoa and Baldasseroni ('38) reported that low doses of Cristallovor, which had no effect upon genetical females, might accelerate the male differentiation in a sexually semi-differentiated race of *Rana esculenta*. In the present study, however, the medulla may be slightly retarded in its development. Differentiation of the gonads of presumably genetical males was not essentially different from that in the controls. Witschi ('50, '53) asserted that the action of the hormones on the gonads is always of inhibitory nature. On the other hand, Padoa ('50) stated that direct stimulation of the medullary development can be found in the masculinization induced by the estrogens. At least in the present case, there was no indication of any direct stimulation of the medullary development, and masculinization seemed to be principally due to the regression of the cortical elements. The effect on the cortex first resulted in the suppression of formation of the oocyte. However, it was not so complete as to prevent restoration of the ovarian nature after early cessation of the treatment. The oocytes existing at the beginning of the treatment could arrive at the auxocyte stage under the hormonal influence.

It is of special interest in this study that in many affected gonads of the tadpoles central cavities were formed between the segmental rete cords by the treatment during the whole first half of the larval period, and that these cavities occasionally opened into the caval vein. Such a structure is quite different from the normals. It seems appropriate to consider that the occurrence of the cavities is consequent upon deficiency of the mesenchyme which is a component of the primary gonadal cavity and develops subsequently into the ovarian interstitial tissue. This effect may be seen already at the stage of the primordial gonad, in consequence of which the cavities are provided with the opening into the caval vein. However, the inhibitory effect on the growth of the mesenchyme seems to be imperfect and variable according to the individuals, as has already been mentioned. Thus it may be suggested that the estrogens in large doses may induce regression of the cortical mesenchyme of the gonads and subsequent inhibition of the oogenesis in various degrees, though the phenomenon here presented needs to be examined by further studies.

### Summary

Administration of 1mg/l of estradiol caused masculinization in the sexually semi-differentiated larvae of *Rana temporaria*. The oogenesis was distinctly suppressed and the primary gonadal cavities were deficient in mesenchyme as a

result of the treatment in early larval period. These inhibitory effects were imperfect and the gonads could recover their ovarian nature if the treatment was ended during the first half of the larval period. Degeneration of the auxocyte was inconspicuous in the present study. It seems probable that the regression of the cortical mesenchyme may play a role in the masculinizing process caused by a large amount of estradiol.

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