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Production of Testis-Ova in Adult Males of *Oryzias latipes* VIII. Effect of Administration of Desiccated Frog Pituitary on Testis-Ovum Production in Males Receiving Estrone Pellet

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In adult males of the fish, *Oryzias latipes*, egg-like cells sometimes appear in the testes following administration of hormonal steroids, especially estrogens (Okada, 1943; Egami, 1955a,b; etc.). In a series of papers, mechanism involved in the formation of the testis-ova has been discussed (Egami, 1955a-e, 1956a,b). The purposes of the present study are to observe the effects of administration of pituitary substance on testis-ovum production in *Oryzias* males.

I wish here to express my cordial gratitude to Prof. K. Takewaki and Dr. Y.K. Okada for their criticism and encouragement.

Experiments

Adult males of the red variety of *Oryzias latipes* were used as materials. Between 1953 and 1956, 4 series of experiments were carried out in different seasons (Table 1). In each series, 3 groups of fish were kept in separate glass vessels containing about 2 liters of water at 23–28°C and fed with freshwater oligochaetes during the course of the experiments.

At the beginning of the experiments, each individual of all groups received a subcutaneous implant of a small estrone pellet. In control groups (Groups A in Series I–III), fish were subjected to no further treatments. In experimental groups, each fish was subcutaneously implanted with about one-fourth of a desiccated pituitary gland of the frog, *Rana nigromaculata*, simultaneously with the estrone pellet (Groups B in Series I–IV) or 7–11 days (Group C in Series I–IV) or 26 days (Group D in series IV) after the pellet implantation. Sacrifices were performed 32–40 days after the pellet implantation in all groups. The testes were fixed in Bouin's fluid and sections were stained with Delafield's hematoxylin.

The testes were divisible into 3 groups according to the degree of development of the testis-ova. The index of testis-ovum production (I) was calculated for each group (Egami, 1955c).

Perusal of Table 1 summarizing the results revealed that the production of testis-ova took place less frequently in the fish receiving simultaneous implanta-

Table 1. Testis-ovum production in *Oryzias* males following administration

| Series | Group | Treatment | | |
|--------|-------|------------------------------|--------------------------------|-----------------|
| | | Date of estrone implantation | Date of pituitary implantation | Date of autopsy |
| I | A | June 23, 1953 | — | July 30, 1953 |
| | B | " | June 23, 1953 | " |
| | C | " | July 2, 1953 | " |
| II | A | Sept. 3, 1955 | — | Oct. 8, 1955 |
| | B | " | Sept. 3, 1955 | " |
| | C | " | Sept. 14, 1955 | " |
| III | A | Feb. 16, 1956 | — | Mar. 19, 1956 |
| | B | " | Feb. 16, 1956 | " |
| | C | " | Feb. 25, 1956 | " |
| IV | B | Oct. 20, 1956 | Oct. 20, 1956 | Nov. 29, 1956 |
| | C | " | Oct. 27, 1956 | " |
| | D | " | Nov. 15, 1956 | " |

tions of the desiccated pituitary and estrone pellet (Group B) than in those given the pellet only (Group A). On the other hand, in those implanted with the pituitary substance 7–26 days after the estrone pellet (Groups C and D) the testis-ova were better developed than in the estrogenized controls (Group A). Even in September (Series II), when implantations of estrone pellet produce testis-ova less frequently than in the rest of the year (Egami, 1956b), the index of testis-ovum production for Group C was very high in comparison with that for the control group (Group A).

Discussion and conclusion

In amphibians, many workers have studied effects of administration of hormonal steroids, singly or in combination with transplantation or extirpation of the pituitary gland, on the differentiation and development of the gonads. However, the results so far reported were not in good agreement. While some authors concluded a participation of the pituitary gland in the gonadal differentiation (Asayama, 1955; Uchida and Hanaoka, 1942, 1949; etc.), others did not subscribe to the view (Mintz and Gallien, 1954; Witschi, 1953; etc.). In adult males of the newt, *Triturus pyrrhogaster*, it was found that testis-ova were frequently produced following injections of estrogens in the regenerating testes of partially castrated males (Uchida and Hanaoka, 1949; Hanaoka, 1950). According to Hanaoka, however, if a pituitary extract was injected simultaneously with estrogen, the production of testis-ova was strongly inhibited in such newts. He also reported, on the other hand, that if the similar injections of the pituitary extract were made a few months after the estrogen administration, testis-ova developed better than in control animals receiving estrogen only.

of desiccated frog pituitary in combination with estrone

| Total number of fish (A) | Number of fish bearing testis-ova | | Index of testis-ovum production $\left(\frac{1/2B+C}{A} \times 100\right)$ |
|--------------------------|-----------------------------------|-------------------------------|---|
| | poorly developed testis-ova (B) | well developed testis-ova (C) | |
| 9 | 2 | 3 | 45 |
| 10 | 1 | 0 | 5 |
| 8 | 2 | 5 | 75 |
| 15 | 3 | 0 | 10 |
| 7 | 1 | 0 | 7 |
| 7 | 2 | 3 | 57 |
| 12 | 3 | 4 | 46 |
| 11 | 2 | 0 | 9 |
| 12 | 4 | 6 | 67 |
| 5 | 2 | 0 | 20 |
| 8 | 4 | 3 | 63 |
| 7 | 2 | 2 | 43 |

In a variety of fishes, although it has been reported by many workers that the testis-ova are produced in the testes of males treated with estrogenic and other hormonal steroids (Berkowitz, 1938; Okada, 1943; Padoa, 1937; etc.), effects of pituitary administration on the phenomena have not yet been investigated.

The results described in the present paper indicate that effects exerted by implantation of desiccated frog pituitary on the production of testis-ova in *Oryzias* males receiving estrone pellets are very different under different experimental conditions. This may be explained on the basis of my previous conclusion that following the administration of estrogen the secretion of gonadotrophins from the pituitary gland of the recipient males is decreased with consequent impairment of the spermatogenic activity of the testis, and that the testis-ova are readily formed in these affected testes during the restoration of spermatogenesis (Egami, 1955 a-e, 1956a,b). If the frog pituitary is implanted simultaneously with estrone pellet, it seems highly probable that the spermatogenesis in the testis is not much affected, since gonadotrophins in the implant compensate to some degree for the decrease in the secretion of gonadotrophins from the pituitary of the recipient fish. It has already been reported that implants of a piece of a desiccated frog pituitary attenuate the inhibitory effects of estrogen upon the ovarian development in female *Oryzias* (Egami, 1954). If, however, the pituitary substance is administered to the fish 7-26 days after estrone pellet implantation, recovery of estrone-damaged spermatogenesis is considered to be hastened. Under such a situation, the testis-ovum production is rather stimulated.

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