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Peculiar Hermaphroditic Indications Found in the Ovary of the Goldfish

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Abstract Four immature adults of goldfish, *Carassius auratus*, were found having ovaries with vestigial testicular tissue. The ovaries were provided with a hypertrophied germinal layer along the inner margin of ovigerous lamellae where oocyte development reaching the primary yolk stage was normally proceeding. The hypertrophied germinal layer was composed of irregularly arranged, large clusters of gonial cells of transitory sizes and clusters of gametogenetic cells presumed to be spermatocytes in respect to their sizes and cytological characteristics. A mitotic division of germ cells was frequently detectable in the gonial cell clusters, while it was hardly seen in oogonial patches of normal ovaries at a comparable stage of oogenesis. Furthermore, repeated occurrence of cell division defined as the first meiosis made it possible to assure that the unusual gametogenesis carried on in the hypertrophied ovarian germinal layer was the spermatogenesis in its nature. The spermatogenesis appeared to fail to progress into spermiogenesis, and was thought to end in degeneration as a result.

Introduction

The occurrence of temporary hermaphrodites in adult teleost fishes has been repeatedly reported in various species as reviewed by Atz (1964). In the goldfish *Carassius auratus*, several cases of hermaphroditic gonad have been studied so far (Kinoshita, 1933; Makino, 1934; Egashira, 1935). The hermaphroditism described in all of these cases was represented by the appearance of oviform germ cells, or the so-called testis-ova, in the testis. So far as the writer knows, no report has dealt with the ovary modified by the existence of vestigial testicular characteristics in teleost fishes of innately gonochoristic nature such as the goldfish.

During the course of histological studies on the gonads of the goldfish, the writer happened to find out fish with ovaries which were furnished with germinal tissue bearing testicular characteristics in terms of histological features, which will be treated with in this report.

Material and method

Until now, four immature goldfish were recorded to have ovaries of the same hermaphroditic tendency. Bodily characters of these fish are indicated in Table 1. Out of those four, three fish (Nos. 1–3), having been the main subjects of the present observations, originally belonged to a group of twenty females which, together with twelve males, were sacrificed in January 1968 at the termination of the experiment designed to search for the effect of pinealectomy and/or bilateral ophthalmectomy on gonadal maturation, each arising in the pinealo-ophthalmectomy group, ophthalmectomy group, and intact control group, respectively. These fish were purchased from a commercial pet fish dealer in Hakodate in July 1967, when they were about 30 mm in body length, and were subsequently raised in an outdoor pond in the faculty campus of Hokkaido University under the natural conditions of light and temperature. In August they were divided into three experimental and one control

Table 1. Bodily characteristics of hermaphroditic goldfish studied.
Figures with asterisks indicate the mean values for 6 fish in the same lot.

Fish No.	Body length (mm)	Body weight (g)	Gonad weight (mg)	Remarks
1	70.0	13.3	100	Pinealo-ophthalmectomized
2	69.4	15.8	340	Ophthalmectomized
3	71.2	14.8	250	Intact control
4	69.8*	14.9*	106*	Normal

groups, then kept in aquaria under water temperature of 20–23°C and natural illumination, during the period of experiment lasting for about 5 months. They were fed on commercial pelleted diet for fishes throughout the period. The surgical operations were done on the fish lightly anesthetized by MS-222.

The remaining hermaphroditic fish (No. 4) was discovered in a group of six normal females examined in September 1969. They were originated from a stock of fish obtained from a commercial dealer in Nara Prefecture and subsequently reared in the outdoor pond.

For histological observations the gonad was fixed in Bouin's fluid or in Heidenhain's Susa, cut at 8 μ in thickness and stained with Delafield's hematoxylin and eosin. As to the terms of oogenetic stages employed in this report, the descriptions made by Yamazaki (1965) may be consulted.

Observations

The most prominent feature of the ovarian anomaly was detected in the gonad of the fish No. 1. At autopsy, the gonad was defined as small immature ovaries based on external appearances, though it was less in weight than that of the other female fish of the same group, the gonosomatic indices (gonad weight × 100/body weight) being 0.75 in the former and averaging 2.00 in the latter. The small ovary was provided with well-formed ovigerous lamellae in which there were many oocytes, 150–200 μ in size, of the early yolk vesicle stage together with smaller ones in the peri-nucleolus stage, being similar in developmental degree to normal ovaries of the other fish of the same group (Fig. 1, 1 and 3).

In normal ovaries of this oogenetic stage, the germinal layer covering the inner margin of ovigerous lamellae was very thin, with dispersed patches of germ cell cysts as notable components. The germ cell cysts were mainly of oogonia of about 12 μ in cellular and 6–8 μ in nuclear diameters, and each of the gonial cysts contained a small number of the germ cells which rarely showed mitotic figures. Cysts of oocytes of the chromatin-nucleolus stage were seen only rarely in the germinal layer (Fig. 1, 2).

The ovary of the fish No. 1 was characterized by the presence of a conspicuously hypertrophied germinal layer spreading over the whole inner surface of ovigerous lamellae, as shown in Fig. 1, 3. The germinal layer was packed with a large number of irregularly arranged clusters of germ cells (Fig. 1, 4). The germ cell clusters were various in size, but were generally much larger in comparison with the cysts of germ cells found in normal ovaries. Many of the germ cells were of the gonial type, each having a round or oval nucleus with a distinct eosinophilic nucleolus. Gonial cells of some transitory sizes were generally noticeable in separate clusters, but those existed as clusters of more than ten cells each in a transection of a cluster, measuring 7–8 μ in cellular and 5–7 μ in nuclear sizes, predominated in the gonial clusters. Besides these, large round gonial cells of 12–13 μ in cellular size with a nucleus of about 9 μ in diameter were occasionally encountered singly or in clusters of two or more cells on the periphery of the germinal

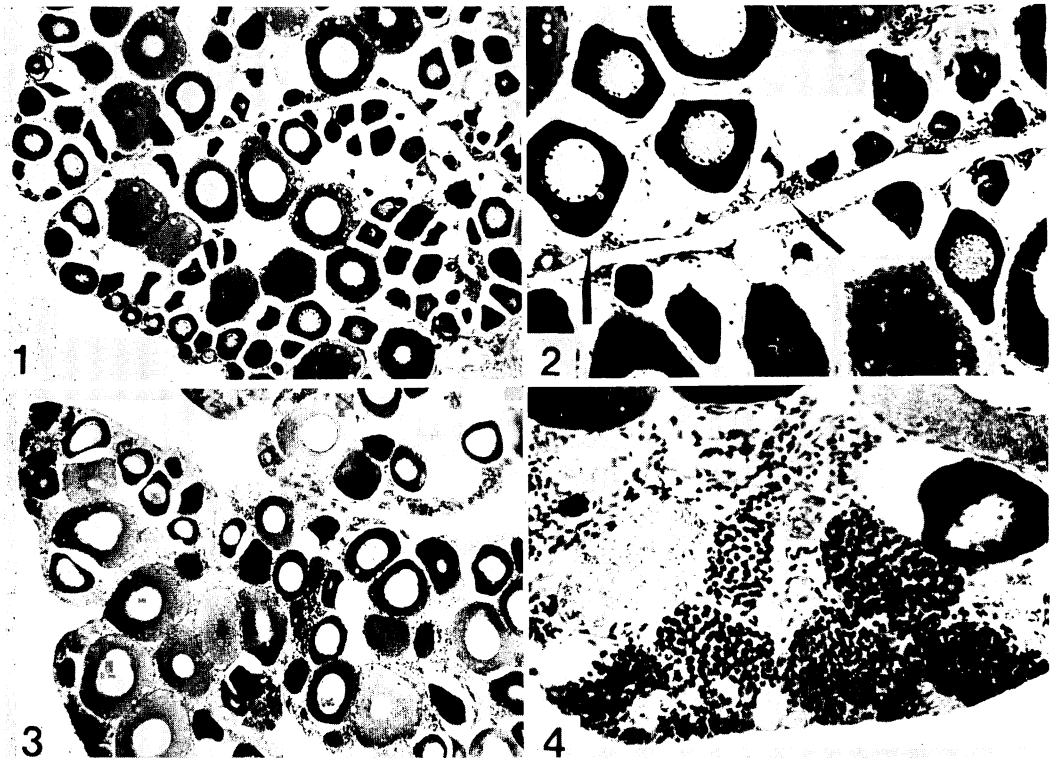

Fig. 1. Photomicrographs of gonads of goldfish studied. 1, A normal ovary of the goldfish in the early yolk vesicle stage of oogenesis. ×52; 2, A part of a normal ovary, showing oogonial patches (arrows) in the germinal layer covering ovigerous lamellae. ×130; 3, An ovarian gonad of the hermaphroditic fish No. 1. ×52; 4, Hypertrophied germinal layer of the gonad of the fish No. 1, indicating the presence of irregularly arranged large clusters of germ cells in various gametogenetic stages. ×260.

layer. Mitotic figures were frequently seen in close proximity to those clusters of gonial cells (Fig. 2, 5).

There also occurred many large clusters of germ cells which appeared to progress in gametogenesis in the thickened germinal layer. The cluster was enclosed in a thin sheet of the cytoplasm of several somatic cells with small elongated nuclei, and was composed of compactly packed germ cells which, on many occasions, were gathered in groups of their respective gametogenetic stages (Fig. 2, 6). One kind of the component germ cells had nuclei of 4–5 μ in diameter in which patterns of condensation of chromatin threads, and the size of the nucleus as well, displayed strong affinity with those of the primary spermatocyte (Fig. 2, 7). Another kind of the chief components consisted of smaller germ cells of about 3 μ in nuclear diameter which strongly resembled the secondary spermatocyte in nuclear cytology (Fig. 2, 8). Furthermore it was evident that some of the cells were undergoing cell division, which was considered to be the first meiotic division because of their frequent appearance in the clusters of the cells defined as primary spermatocytes (Fig. 2, 9).

The above-described characteristics of germinal components in the hypertrophied germinal layer show striking resemblance to the spermatogenetic cells in normal testis. In the present case, however, no formation of testicular tubules was discernible, and no signs of successful spermiogenesis were detectable. In addition, not all of the germ cells included in a cluster were carrying on gametogenesis at a synchronous pace, in

contrast to the process of normal spermatogenesis in a germ cell cyst in the testicular tubule. It was further noticed that some cells in the germ cell clusters were frequently seen to undergo degenerative changes (Fig. 2, 10).

In spite of unusual hyperplasia of young germ cells, cysts of normal oocytes of the chromatin-nucleolus stage and those of the initial peri-nucleolus stage were repeatedly observed intermingled with the hypertrophied

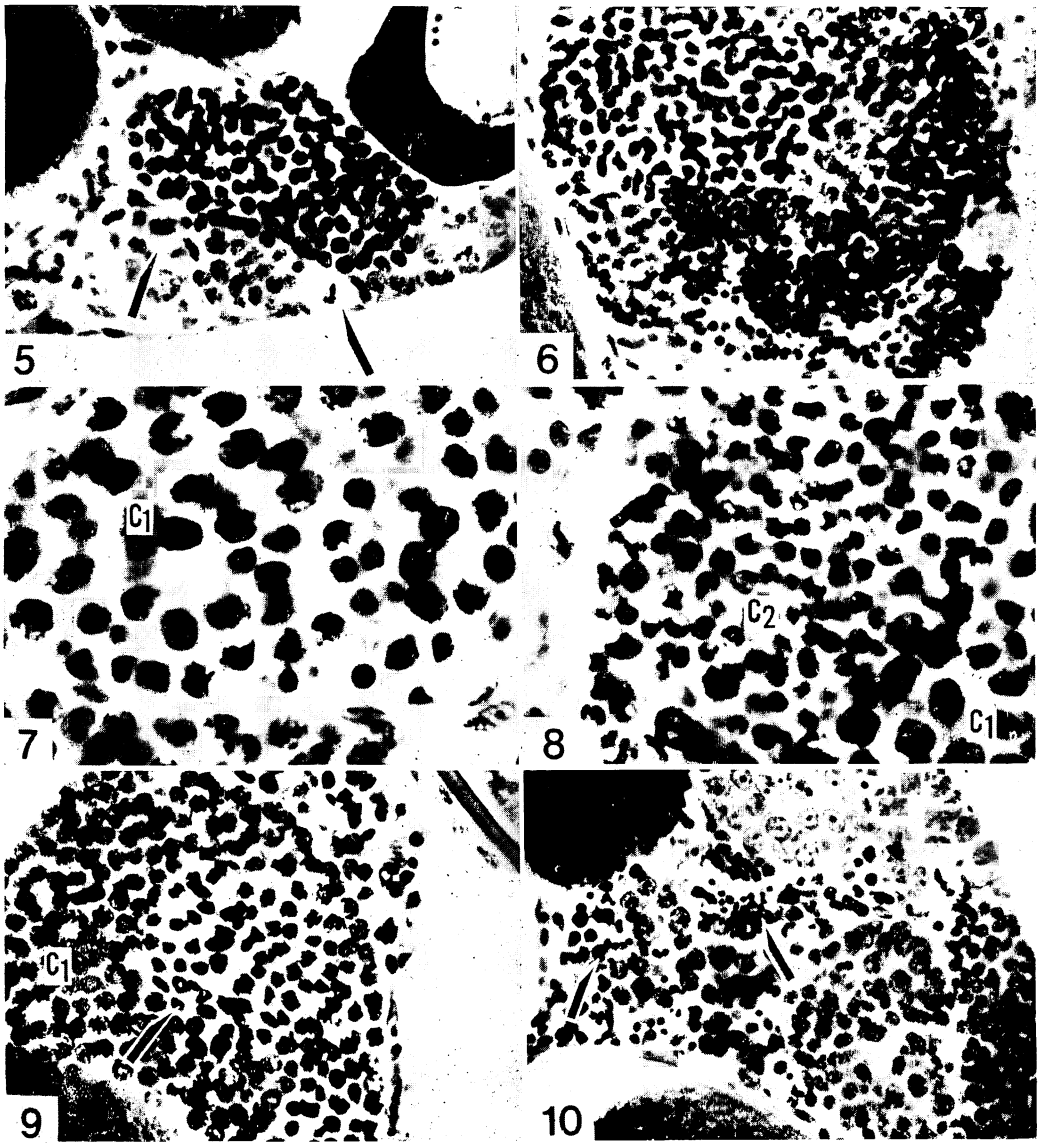

Fig. 2. Photomicrographs of gonads of goldfish studied. 5, Mitotic division (arrows) of gonial germ cells in the hypertrophied germinal layer of the fish No. 1. ×520; 6, A hyperplastic cluster containing several groups of germ cells of different gametogenetic stages in the fish No. 1. ×520; 7, A cluster of germ cells bearing cytological characteristics of primary spermatocytes (C_1) in the fish No. 1. ×1,280; 8, A germ cell cluster containing the cells defined as secondary spermatocytes (C_2) in the fish No. 1. ×1,280; 9, A cluster of primary spermatocyte-like cells (C_1), with those undergoing meiotic division (arrow) in the fish No. 2. ×520; 10, Clusters of germ cells, revealing degenerating cells (arrows) in some of them in the fish No. 1. ×520.

germ cell clusters in the germinal layer. No signs of degeneration were detected in oocytes of every oogenetic stage insofar as the present observation was concerned.

Ovaries with the same pattern of gametogenetic modification in the germinal layer as mentioned above were observed to exist also in the fish Nos. 2 and 4. In the fish No. 2, especially, oocytes were progressing into vitellogenesis to attain the size of 500–600 μ and to reach the primary yolk stage just as those present in ovaries of the other four females of the same group, the gonosomatic indices being 2.15 in the former and 2.40 in average in the latter. Nevertheless the germinal layer was thickened by the development of many and large clusters of gonial and spermatogenetic cells, though the hypertrophy was rather slight in degree than that seen in the ovary of the fish No. 1. This fact may denote that the gonad in question is the ovary in its inherent nature and may be capable of developing into maturation. Ovaries of the fish No. 3 were a little less in weight than those of the other females of the same group, but were similar in degree to the oogenetic development of the latter. The ovary was modified by the existence of a thick germinal layer in which clusters of gonial germ cells were packed. Clusters of the germ cells of the type of spermatocytes were seen rather occasionally in this case.

Discussion

Diversity of sexual development in teleost fishes has been extensively reviewed and discussed by Forbes (1961), Atz (1964), and others. As these authors stated, the diversity seems to be at least in part attributable to the lack of the medullary component, the somatic element which ensures male differentiation of associated germ cells in the gonad of the other vertebrate groups (Witschi, 1957), in the gonad of teleost fishes. The resulted dominancy of the region homologous in embryological origin to the cortex, which is capable of inducing female differentiation of allied germ cells, in the fish gonad might account for relatively labile nature encountered in testicular differentiation. Such seems to be also the case in the goldfish. Stromsten (1931) mentioned, though only briefly, about hermaphroditic tendencies in testicular development of the goldfish. The appearance of degenerative oviform germ cells, or the testis-ova, in the testis of adult fish is not rare in the goldfish examined by the writer.

The ovaries of the four goldfish reported herein are clearly demarcated from normal ones by distinct histological modifications in the germinal layer along the inner surface of ovigerous lamellae. The modifications are shown in the following two features: (1) increment in number and hypertrophy of clusters of gonial cells, in possible association with activated mitosis of the cells; (2) remarkable existence of large clusters of germ cells in some stages of gametogenesis in the hypertrophied germinal layer. Of the two, the second feature is of a peculiar interest, for the germ cells in gametogenesis bear the cytological characteristics of primary and secondary spermatocytes. An occurrence of meiotic division in the germ cell clusters may further support the view that the hypertrophied germinal layer may be considered to be the testicular tissue, since in the case of oogenesis the primary oocytes commence their growth into those of vitellogenetic phases at the diplotene stage of meiotic prophase. The present case seems to be a new type of naturally occurring hermaphroditic gonad in gonochoristic teleosts.

The testicular tissue seems to be vestigial in functional nature, for the arrangement of the germ cell clusters is quite irregular throughout the hypertrophied germinal layer, and the presumed secondary spermatocytes do not go through spermiogenesis but undergo degeneration. It is still unknown whether the degeneration results from a certain inhibitory influence of a predominating ovarian tissue or from a lack of a certain factor(s) which successfully induces normal spermiogenesis

in the testicular tissue. Anyhow, it is highly probable that the hermaphrodites are innately the females in gonadal sex, although no functional evidence for the ovary has been gained concerning the hermaphroditic gonad in question.

The cause of occurrence of the testicular tissue in the ovary remains quite uncertain. It seems to be impossible to attribute the cause to the effect of surgical operations, since the modified ovary was found also in the goldfish free from such operations. Moreover, pinealectomy and/or ophthalmectomy have been proved to be without effect on gonadal development and maturation in the goldfish (Peter, 1968; Takahashi, unpublished). In some fishes such as *Xiphophorus hellerii* and *Lebistes reticulatus*, fungal infestation may sometimes be responsible for gonadal masculinization (Wurmbach, 1951). The goldfish examined in the present study showed, however, no signs of parasitic diseases in the external appearances of visceral organs nor in the histology of the gonad at the time of autopsy.

A clue for explaining the occurrence of the peculiar hermaphroditic structure in the ovary must be sought in the mechanism of gonadal sex differentiation during larval stages. It has been clearly demonstrated that, in the goldfish as well as other animals, germ cells in the sexually indifferent gonad are bipotential in terms of sex differentiation (Yamamoto and Kajishima, 1968). Is there any histological basis, like the gonadal cortex and medulla in the other vertebrates, which participates in the induction of sex differentiation of such germ cells in the developing gonad of teleost fishes? In this respect, Miyamori's (1961, 1964) descriptions about the behaviour of testicular somatic cells in *Lebistes reticulatus* are quite interesting: the somatic cells which aggregate at the gonad hilum at an initial stage of gonadogenesis proliferate to form efferent duct and to surround the clusters of gonia during testicular differentiation; their development is entirely suppressed by treatment with estrogen whereas it is promoted by androgen, imitating the response of the medullary cells in amphibian gonad to the hormones (Takahashi, 1959). Although gonadal somatic cells in the course of sex differentiation of the goldfish are not so prominent in their behaviour as in the guppy, they appear to show a certain characteristic pattern of development during testicular morphogenesis, which will be reported later. An androgenic inductor system of gonadal sex differentiation must not necessarily be the medullary tissue of mesonephric blastema origin, but might be composed of somatic cells of different origin in teleost fishes. Both the medullary and the somatic cells mentioned above are believed to be potentiated to produce androgens in the differentiated testis (Chieffi, 1966; Yaron, 1966).

It seems likely to occur that such an androgenic inductor system has happened to be maintained as vestiges in the developing ovary and has exerted an incomplete androgenic influence upon ovarian primordial germ cells which are still bipotential in sex differentiation. At present this is only a matter of speculation, and further accumulation of various cases of hermaphroditism, and of detailed observational results on natural and experimental sex differentiation as well, is needed to substantiate the speculation.

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キンギョの卵巣にみられた特殊な雌雄同体的構造

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キンギョ生殖腺の発達の組織学的研究の過程で卵巣に痕跡的精巣組織をもつ雌成魚4尾を見出した．これらの卵巣では卵形成は正常に進行しており，その進行の度合は同系の正常雌魚の卵巣と差がなかった．しかし卵巣腔に面する生殖上皮は，正常卵巣ではごく少数の静止期卵原細胞巣が散在するのみであったが，この4尾の卵巣では巨大かつ多数の生殖細胞集塊の不規則かつ密な配列によって著るしく肥厚していた．この集塊のあるものは種々の移行的な大きさのゴニア型の細胞より成り，活発な増殖分裂が認められた．また他種の集塊は，その大きさおよび核の細胞学的特徴から，第一および第二精母細胞とみなしうる細胞を含み，さらに減数第一分裂中期と推定される分裂像を示す細胞をも有していた．しかし精細胞および精子の形成は観察されず，精子形成の中断を示すと思われる退化途上の細胞集団がみられた．このように，この4尾の卵巣生殖上皮の異常が痕跡的精巣組織の残存によることが確かめられた．この型の生殖腺形態異常は本来雌雄異体性の魚種に偶発する雌雄同体性の新型と思われるが，その発現の原因は不明である．

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