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STUDIES ON THE MATURATION OF SALMONID FISHES-I. CHANGES
IN THE TESTIS OF THE CHUM SALMON, *ONCORHYNCHUS*
KETA, DURING ANADROMOUS MIGRATION

Osamu HIROI* and Kiichiro YAMAMOTO*

Among salmonid fishes, the species belonging to the genus *Salmo* which perform annual reproduction have hitherto been rather well studied concerning the maturational process of the testis (Jones, 1940, in *S. salar*; Weisel, 1943, Robertson, 1958, and Oota *et al.*, 1965, in *S. gairdnerii irideus*). Those in the genus *Oncorhynchus*, which are destined to die soon after their first mating, have also been the subjects of a few researches on the maturation of testes. Weisel (1943) made excellent histological observations on the testicular maturation of *O. nerka*, but his material was limited to a landlocked form of the fish. Ishida *et al.* (1961) described also the annual cycle of kokanee testes.

About *O. keta*, Ishida *et al.* (1961) examined histologically the testis of immature chum salmon captured in the northern Pacific Ocean, but the detailed descriptions about germ cells and other components of the testis were not given. Furthermore, no investigations have been done so far on the maturational process of the testis of salmonid fishes during their anadromous migration to their spawning ground. Thus the authors' interest is in pursuing detailed histology on the developmental course of the testis from the immature to the fully mature state in salmonid fishes, taking their habits of reproduction in comparison. This paper reports some histological observations on the maturing testis of the chum salmon, *O. keta*, caught mainly during their anadromous migration.

Before proceeding further, we wish to express our hearty thanks to Dr. Seizo Sano, Mr. Kazuhiko Nishino and Dr. Toyohiko Hikita, Hokkaido Salmon Hatchery, and to Messrs. Shigeru Hara and Yoshio Ishikawa, the Tokachi Branch of Hokkaido Salmon Hatchery, for their friendly facilities in the collection of the present material. Thanks are also due to the Japanese Conservational Society of Fisheries Resource for the grant of search fund.

Material and Methods

Males of the chum salmon *Oncorhynchus keta*, 27 fish in total, were captured at three points on the route of their anadromous migration in the Tokachi River, eastern Hokkaido, during the period from September to October, 1966 and 1967,

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and were used as a material in the present study. In addition to these specimens two fish caught in the northern Pacific (45°N., 158°E.) in April, 1968, were also examined.

Some of the fish caught in the coastal sea off Atsunai, about 20 km distant from the mouth of the river, already had a tinge of purplish red in body color, while others still looked to be silvery. The fish arrived at the mouth of the river were captured at Ōtsu, where the river water was known to be brackish. The salmon on the midway of their anadromous migration were collected at Chiyoda, about 45 km from the mouth of the river. The fish which showed ejaculation of milt when being pressed gently on the abdomen were regarded as mature fish, and those which failed to excrete the milt were recorded as maturing ones. Based on the localities of sampling and the degree of maturity determined grossly, the specimens used in the present study were divided into six groups, as indicated in Table 1.

Table 1. Characters of the sampled groups of *O. keta* collected during the anadromous migration

Group	Locality of capture		Date of capture	Number of specimens collected	Remarks
I	158°E 44°N	Northern pacific (sea water)	Apr. 20 '68	2	Immature
II	Atsunai	Coastal sea (sea water)	Sep. 27 '67	2	Maturing, silvery in color
III			Oct. 17 '67	1	
			Sep. 27 '67	3	
IV	Ōtsu	Mouth of river (brackish water)	Sep. 29 '66	5	Maturing, with nuptial coloration
			Sep. 28 '67	2	
V	Chiyoda	Mid-stream (fresh water)	Sep. 29 '66	4	
			Sep. 25 '67	2	
VI			Oct. 12 '66	4	Mature, with nuptial coloration
			Oct. 16 '67	2	
(Total)				27	

For histological observations, the testes were removed, measured in weight, cut into small pieces and fixed in Bouin's fluid or Zenker-formol solution. Serial paraffin sections of the testes were cut 5 to 6 micra in thickness and stained with Delafield's hematoxylin-eosin, with Heidenhain's iron hematoxylin-light green or with Heidenhain's azan.

In order to determine the maturity of testes, three histological sections of each testis were transcribed on tracing papers by the aid of a magnifying

projector. Spaces occupied by mature spermatozoa were separated from the other germinal portion by cutting the drawings, and measured respectively in weight after discarding the part of traced blood vessels. Then the testicular maturity was determined in terms of percent ratio of the weight of the portion of mature spermatozoa to that of the total germinal portion of the testis in each fish.

Observations

I. *Histological changes in the testis of the salmon during anadromous migration*

Maturing testes of the chum salmon are situated dorsal to the gut and ventrolateral to the air bladder as paired, elongate bodies which are opaque and milky white in color. A short gonoduct starts from the posterior end of each testis, and these ducts unite to form a common duct which opens on the urogenital papilla.

As shown in Table 2, histological survey on testes of the fish collected fairly indicates that the maturity of testes in terms of spermatogenetic phases proceeds with the progress of the anadromous migration of the salmon. In the testes of fish of group I, which were captured in the northern Pacific, all germ cells in

Table 2. Germ cell components found in the testis of each fish in the respective sampled groups of *O. keta* captured during the anadromous migration

Group	Number of fish examined	Cyst of spermatogonia	Cyst of spermatocytes	Cyst of spermatids	Spermatozoa
I	2	+			
II	3	+	+	+	+
		+	+	+	+
		+	+	+	+
III	3	+	+	+	+
		+	+	+	+
		+	+	+	+
IV	6	+	+	+	+
		+	+	+	+
			+	+	+
			+	+	+
			+	+	+
V	5	+	+	+	+
			+	+	+
			+	+	+
			+	+	+
			+	+	+
VI	6		+	+	+
			+	+	+
				+	+
				+	+
				+	+
				+	+

The mark, +, shows the germ cell present in testicular lobules of each fish.

testicular lobules are spermatogonia during the resting or mitotic phase, being in the multiplication stage (Fig. 14). The spermatogonia are found singly or in clusters of several cells. Spermatogonia present singly in lobules are generally the largest of all germ cells in size. Each spermatogonium is surrounded by a flat, somatic cell which may subsequently develop to form a cyst wall. The germ cells in the resting phase, 10–14 μ in size, have very distinct cytoplasm surrounding a large, round or elliptical nucleus of 9–12 μ in diameter which contains usually one nucleolus and several chromatins stained deeply with hematoxylin (Fig. 1). On the other hand, those of the clustered form have an indistinct cytoplasm and a round nucleus of various diameters (5–9 μ) which contains two or three nucleoli and sparse chromatins stained deeply with hematoxylin (Fig. 2). More than one spermatogonium are generally seen in the mitotic phase throughout the testicular lobules (Fig. 3), though they are in some cases grouped as a cluster of several dividing cells (Fig. 4).

On the other hand, in fish of groups II and III, which were captured in the coastal sea, the germ cells of every maturational stage are seen in the testicular lobules, though no spermatogonia in the mitotic phase are detectable (Fig. 15). Spermatogonia in these fish are generally found making a cyst of several cells (Fig. 5), though in some cases single spermatogonium is also present along the wall of lobule. They are provided with very indistinct cytoplasm around a round nucleus of 5 μ in diameter, which contains sparse chromatins and two or three nucleoli stained deeply with hematoxylin. The cells seem to have got through the multiplication stage, since the mitotic figure is no longer detectable in the cyst. A transformation of these cells into primary spermatocytes is begun by the aggregation of the chromatins into one pole within the nucleus. The cells become indistinct in boundary and the nucleoli disappear, while the chromatins appear to be fused with each other, occupying a half sphere of the nucleus (Fig. 6). Subsequently, the chromatins develop to form thick threads which soon extend uniformly over the nucleus (Fig. 7). During the period of the transformation into spermatocytes, the cells appear to show no notable change in their size, though the nucleus tends to become large in size (5.5 μ). In the secondary spermatocytes following the first maturation division (Fig. 8), a nuclear wall becomes very distinct, whereas the cell contour remains still obscure. The nucleus becomes smaller in size (4 μ) than in the former stage (Fig. 9). Spermatids following the second maturation division (Fig. 10) possess a distinct, round nucleus which is smaller in size (2.8 μ) than that of the secondary spermatocyte and show an appearance like that of the spermatogonium in the resting phase, except the indistinct cytoplasm (Fig. 11). Afterwards, the stainable nuclear components gather gradually in one pole of the cells into a globular or oval mass which constitutes the head of a spermatozoon (Fig. 12).

Although the salmon caught in the coastal sea do not eject the milt by keeping a slight pressure on their abdomen, mature spermatozoa have already existed in the lobules of these fish (Fig. 15). The amount of mature spermatozoa shows an obvious increase as the migration advances (Figs. 16 and 17).

In general, different germ cell cysts in a testicular lobule are in different spermatogenetic phases, but germ cells within a cyst show a synchronizing progress of spermatogenesis. Such a synchronization is also the case in the development of testicular lobules, every one of those in a testis being provided with the same set of germ cell cysts in various spermatogenetic phases. Thus the histological picture of one of the lobules is able to represent the typical maturational phase of the testis.

Furthermore, it is worthy to note that the cyst of spermatogonia is in no case noticed in testes of the fish of groups V and VI (Figs. 16 and 17), though a few spermatogonia somewhat deformed are found singly on the wall of lobules. All cysts of the spermatogonia are sure to develop into spermatocytes, all of which in turn into spermatids and finally into mature spermatozoa. Thus the variance of spermatogenetic phases among germ cell cysts becomes reduced as testicular maturation advances, as indicated in Table 2.

The spermatozoon of the present species has an oval head, $2.0 \times 2.5 \mu$ in size and stained deeply with hematoxylin, and a long, slender tail. Mature spermatozoa are seen aggregated in the lumen of testicular lobules without any regular arrangement (Fig. 13). They begin to aggregate in the lumina even at the early stage when many of the germ cells still remain immature (Figs. 15 and 16). They do not reveal any connection with the intralobular somatic cell elements, or Sertoli cell homologues, in contrast with the condition known in mammalian testis in which spermatozoa have a close morphological connection with Sertoli cells (Bloom and Fawcett, 1964). In immature testicular lobules which contain many germ cell cysts of early spermatogenetic stages, sperm heads are stained blue with Heidenhain's azan after Bouin's fixation. In testicular lobules occupied almost exclusively by mature spermatozoa, however, they become stained red with the same methods. This may probably mean that the spermatozoa change their physical or chemical nature during the period of testicular maturation. Further examinations seem to be needed to determine whether the spermatozoa stained blue possess normal fertilizability or not.

In sexually immature testes, such as those of groups I, II and III, testicular lobules are completely separated from each other by a thin connective tissue layer in which blood capillaries are finely distributed (Figs. 14 and 15). As testicular maturation proceeds, some of these lobules become to be fused with each other to make a far smaller number of larger lobules. The occurrence of the fusion of testicular lobules is confirmed first in some of the fish of group IV, in

which the testes are completely deprived of spermatogonial cysts. The fusion seems to originate from the union of adjacent walls of the lobules at the point where germ cell cysts have ruptured causing the release of spermatozoa from the cysts into the lumen (Fig. 18). With the progress of sperm formation the lobules continue to fuse into larger ones than before. Thus fully mature testes come to be composed of a small number of huge lobules packed with mature sperms (Fig. 17).

Testicular lobules are consistently lined by a layer of cells with a cytoplasm of indistinct contour, which are closely adherent to the connective tissue surrounding the lobule. These cells seem to coincide in histological aspects with the "lobule boundary cells" described in many teleosts (Marshall and Lofts, 1956). In maturing testes in which spermatogonial cysts are still maintained in the lobules, the cells have nuclei of round, or elliptical shape and of 2×4 to $7 \times 14 \mu$ in size, and those close to the cyst of spermatids are noticed to contain a few small vacuoles of $3-5 \mu$ in diameter in their cytoplasm. As maturational changes proceed in the lobule, those cells with vacuolar inclusions become markedly increased in number, keeping close association with the cysts of spermatids, and further the vacuoles become various in size ($3-15 \mu$) (Fig. 19). After the lobule has been filled with mature spermatozoa, the cytoplasmic vacuoles are still prominent in the cells in spite of a considerable decrease in number. At that stage, however, the nuclei of these cells are clearly smaller in size ($1.5 \times 4 \mu$) than those of the maturing testis and they are mostly of rod in shape.

Testicular interstitial cells existing in the interlobular spaces appear to reveal some changes during the anadromous migration of the salmon but not so marked. Generally, their nuclei with one or two nucleoli are round in shape and about 5.5μ in size and show a comparatively strong affinity to hematoxylin. Their cytoplasm is wide and irregular in contour (Fig. 20).

II. *Changes in the testis weight, maturity factor and testicular maturity during anadromous migration*

The mean values of body length, body weight, testis weight and of the maturity factor in group I which comprises young and sexually immature fish caught in the northern Pacific are far smaller than those in the anadromous fish, being 50.4 cm, 1.6 kg, 1.5 g and 0.1%, respectively (Table 3). Changes in the body length of the male salmon are not significant during the periods of anadromous migration, while those in body weight show a consistent tendency toward increase until the fish arrive at the mouth of the river and a gradual decrease in association with the progress of the anadromous migration (Table 3). The decrease in body weight may be ascribed to the fact that the present salmon do not take food during the period of anadromous migration. The weight of testes undergoes progressive

Table 3. Body length, body weight, testis weight, and the maturity factor (testis weight \times 100 / body weight) of the male salmon captured during their anadromous migration

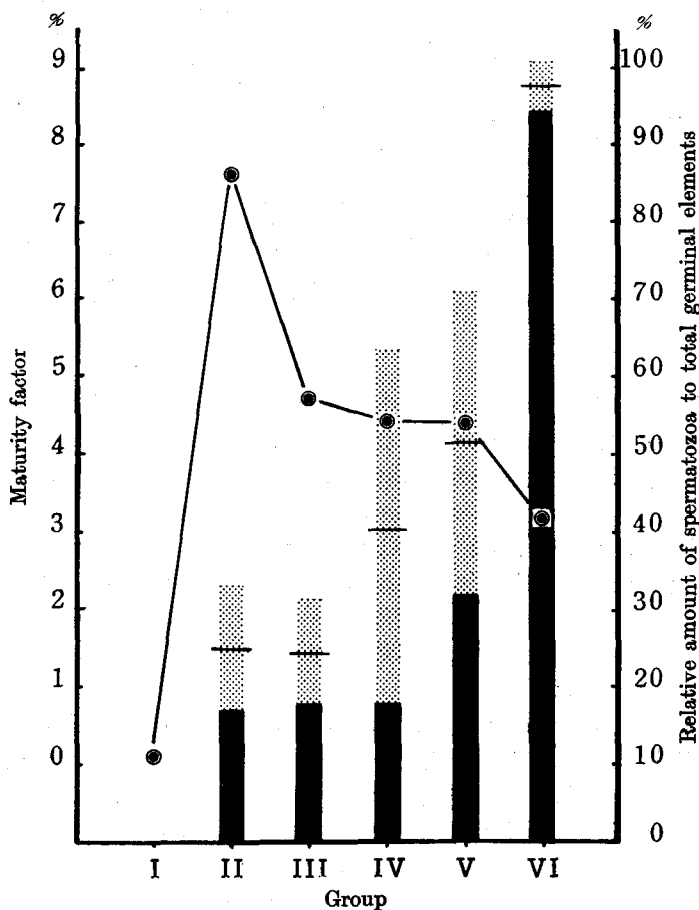
Group	Number of specimens examined	Body length (cm)	Body weight (kg)	Testis weight (g)	Maturity factor (%)
		Mean (Range)	Mean (Range)	Mean (Range)	Mean (Range)
I	2	50.4 (50.3-50.5)	1.6 (1.5-1.7)	1.5 (1.0-2.0)	0.1
II	3	63.8 (53.0-74.0)	3.2 (1.9-4.6)	243 (150-380)	7.6 (6.7-8.3)
III	3	76.0 (71.5-78.5)	4.5 (3.5-5.9)	200 (180-220)	4.7 (3.4-6.3)
IV	7	75.3 (65.4-82.2)	5.4 (3.5-7.1)	224 (170-326)	4.4 (2.3-6.2)
V	6	72.5 (67.6-81.7)	4.9 (2.7-6.9)	197 (153-216)	4.4 (3.0-5.6)
VI	6	74.0 (65.5-82.5)	4.0 (2.5-6.1)	135 (70-212)	3.2 (2.3-4.2)

reduction after the fish have entered the river, as is the case of body weight.

As described in the preceding section, spermatozoa within testicular lobules of the present species increase gradually in number during the anadromous migration. Such a character of sperm formation, together with that of the "total synchronism" type, makes it admissible to adopt the relative amount of spermatozoa to total germinal portion in the testis as a value representing the degree of testicular maturity of this salmon. Changes in the values of testicular maturity during the anadromous migration, and those in the mean value of maturity factor as well, are revealed in Text-figure 1.

The maturity factor during the anadromous migration is found to be the largest (7.6%) in group II in which the value of the testicular maturity is $24.8 \pm 8.4\%$, and is the smallest (3.2%) in group VI which comprises fully mature fish, being $97.6 \pm 3.4\%$ in testicular maturity. In group III, the factor, being 4.7%, is definitely low as compared with that of group II, while the testicular maturity, being $24.6 \pm 6.6\%$, is almost the same as that of group II. This seems to be mostly due to the marked increase in body weight in the former group with no momentous changes in testis weight in comparison with the latter (Table 3). The maturity factors in groups IV and V, averaging 4.4% in both groups, are slightly lower than in group III, whereas the values of the testicular maturity in the former groups, $40.5 \pm 22.8\%$ and $51.5 \pm 19.5\%$, respectively, are found to be clearly large as compared with the latter group. The maximum decrease of the factor found in group VI may be partly attributable to the fact that the fish have already begun to release the milt, since the ejaculation of milt is confirmed in these fish by pressing the belly as described before. The testicular maturity proceeds obviously in this group, the value being about twice as large as that of group V.

The decrease in the value of the maturity factor in harmony with maturational changes in male fishes has been reported in the flounder by Yamamoto (1953), and in the rainbow trout by Oota *et al.* (1965). As suggested by these researchers this decrease in testicular weight may be due to certain physiological changes within the



Text-figure 1. Relation between the change in the maturity factor and that in the testicular maturity during the anadromous migration of *O. keta*

The shaded portion of each column represents 95% confidence limits of values of the testicular maturity. Mean value of maturity factor is shown by the mark ⊙.

testis which may occur prior to the lobular maturation, rather than to the gradual elimination of the semen from maturing males.

Discussion

In many teleost fishes which show cyclic reproduction such as the guppy (Miyamori, 1964) and the plaice (Barr, 1963), the testis always retains some clusters of spermatogonia in the resting stage, the "residual" spermatogonia, for the next crop of spermatocytes. Such is also the case in *Salmo gairdnerii* (Weisel, 1943, and Oota *et al.*, 1965), one of the salmonids which perform annual mating. The condition seems, however, considerably different in the species belonging to the

genus *Oncorhynchus*, in which males and females are both destined to die after having accomplished the spawning. Weisel (1943) reported, in the landlocked form of *O. nerka*, that the resting spermatogonia for a next generation remained through the whole spermatogenetic process along the sides of lobules, but they become vacuolated during the spawning period and then underwent pycnosis. Although such degeneration of spermatogonia have been indistinct in the testes of *O. keta* so far as the present authors examined, spermatogonia of somewhat deformed nature are observable in the fully mature testis of the present salmon.

Mature spermatozoa reveal an interesting behavior after having been liberated from the ruptured cyst. Even in early stages of testicular maturation the spermatozoa are seen aggregated irregularly in the center of the lobular lumina separated from the peripheral germinal layer of each testicular lobule, as is also the case in the rainbow trout (Oota *et al.*, 1965) and in the flounder (Yamamoto, 1953). On the other hand, it is known that spermatozoa of the spotted mackerel, *Pneumatophora tapeinocephalus* (Bleeker), are gathered in bundles arranged in regular direction (Tanoue, 1966), and that those of the viviparous percomorph fish, *Embiotoca jacksoni*, are arranged in "bouquets" within a cyst with their heads oriented outward and their tails embedded in a central mass of hyaline, PAS-positive material (Lagios, 1965). In higher vertebrates, the sustentacular cell of Sertoli is known to play an important role to maintain mature spermatozoa in the testis (Witschi, 1956, Gorbman and Bern, 1962, and Bloom and Fawcett, 1964). In the testicular lobules of the chum salmon, maturing germ cells are embedded in the cysts surrounded by a cytoplasmic sheet which may be a part of the sustentacular cells of Sertoli cell homologue. As maturational changes proceed, the cysts come to rupture and release spermatozoa into the lobular lumen in which the spermatozoa are aggregated into a cluster being completely freed from connection with the intralobular somatic cells. When testicular lobules have been packed with mature spermatozoa, there remains in the lobule an unlayered lining of cells, which seem to be identical with the "lobule boundary" cells described by Lofts and Marshall (1957) in the pike, *Esox lucius*. The method used in the present study is unable to demonstrate decisive morphology of the intralobular somatic cells, and fails to trace their changes following the release of spermatozoa. It seems highly probable, however, that the intralobular somatic cells happen to remain at least as a constituent of so-called lobule boundary cells in mature testicular lobule. The cells of the chum salmon show cytoplasmic vacuolization during the period of active spermiogenesis. Similar vacuolization of the follicle cells, as well as that of spermatogonia and epithelial cells of sperm ducts, has been found in *O. nerka* during the period of spawning (Weisel, 1943). The vacuolization of the lobule boundary cell has been described in the pike by Lofts and Marshall (1957). They showed that such vacuoles were lipid droplets which

were dissolved out during the wax-embedding and many of the lipid droplets were cholesterol-positive. Similar phenomena have been noticed in other teleost fishes such as the cyprinodont fish, *Fundulus heteroclitus*, (Lofts *et al.*, 1966), the viviparous percomorph fish, *Embiotoca jacksoni*, (Lagios, 1965) and the ovoviviparous fish, *Sebastes paucispinis*, (Moser, 1967). From the lipid droplet-cycle they emphasized that the lobule boundary cells transform into the glandular cells of Leydig-cell homologue. The development of glandular nature in the lobule boundary cells has been pointed out also by Marshall and Lofts (1956), and Gorbman and Bern (1962). The lobule boundary cells of the chum salmon show cytoplasmic vacuolization in the late period of spermatogenesis. Further the occurrence of cytoplasmic vacuolization of the cells of lobule boundary in nature appears to coincide with the progress of the metamorphosis of spermatids into spermatozoa in the adjacent germ cell cysts. It remains, however, to be solved whether the vacuolization may reflect the glandular function of these cells or may be an expression of phagocytotic activity which may highly develop in these cells during spermiogenesis.

In the chum salmon, interstitial cells of Leydig-cell homologue are clearly detectable within the interlobular spaces of all the testes examined, and are always discriminated morphologically from intralobular somatic cells. The former cells appear to reveal some changes during spermatogenesis but not so marked.

Further studies are needed to clarify the obscurity about the physiological nature of the somatic cells in connection with germ cell maturation. Electron-microscopical observations are being done by the present authors on the maturing testis of the chum salmon.

Summary

1. Changes in the testes of the chum salmon, *Oncorhynchus keta*, during the anadromous migration are investigated in the present study.
2. The course of maturation in the present species processes synchronously in all of the germ cells within a cyst. Although phases in the spermatogenesis are generally different from cyst to cyst in a testicular lobule, the maturation of the testis seems to be advanced quite synchronously in all the lobules.
3. The testes have already been maturing at the time of commencement of anadromous migration: germ cells of every maturational stage from primary spermatocytes to mature spermatozoa are seen in these testes, while spermatogonia have passed through the multiplication phase at that time. The spermatogonia, spermatocytes and spermatids become indetectable in turn within their testicular lobules associated with the progress of anadromous migration, though a few spermatogonia bearing some deformation are occasionally seen in mature testes.
4. Spermatozoa are accumulated in the lumen of the testicular lobules with-

out any regularity in arrangement, being separated by a narrow space from the periphery of the lobule.

5. The intralobular somatic cells lining the lobule wall show cytoplasmic vacuolization during the period of active spermiogenesis, suggesting the development of glandular nature in these cells. The interstitial cells of the testis appear to reveal some changes during the anadromous migration but not so marked.

6. The testis tends to decrease in weight during the course of anadromous migration before they reach functional ripening. The testicular maturity represented in terms of the relative amount of mature spermatozoa to total germ cells shows a marked increase in value with the progress of the anadromous migration, indicating a rapid maturation of the testis in the fresh water stages of the migration.

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Explanation of Plates

All figures are photomicrographs obtained from sections through testes of immature, maturing and mature chum salmon. Fixed in Bouin's fluid and stained with Heidenhain's hematoxylin and light green

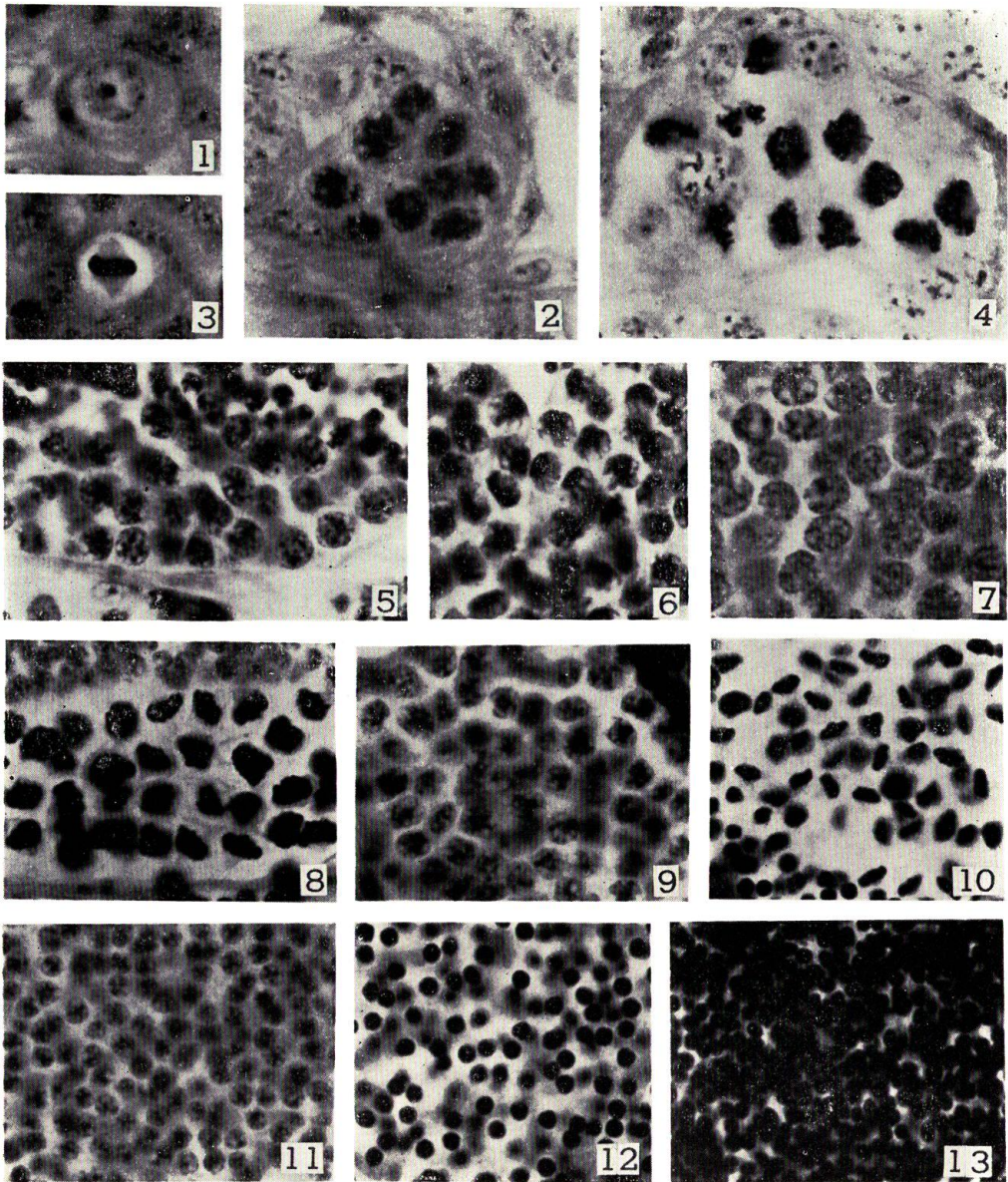
PLATE I

Figs. 1-4. Sections through testes of immature fish caught on April 20 in the northern Pacific (group I). Magnification, $\times 1000$

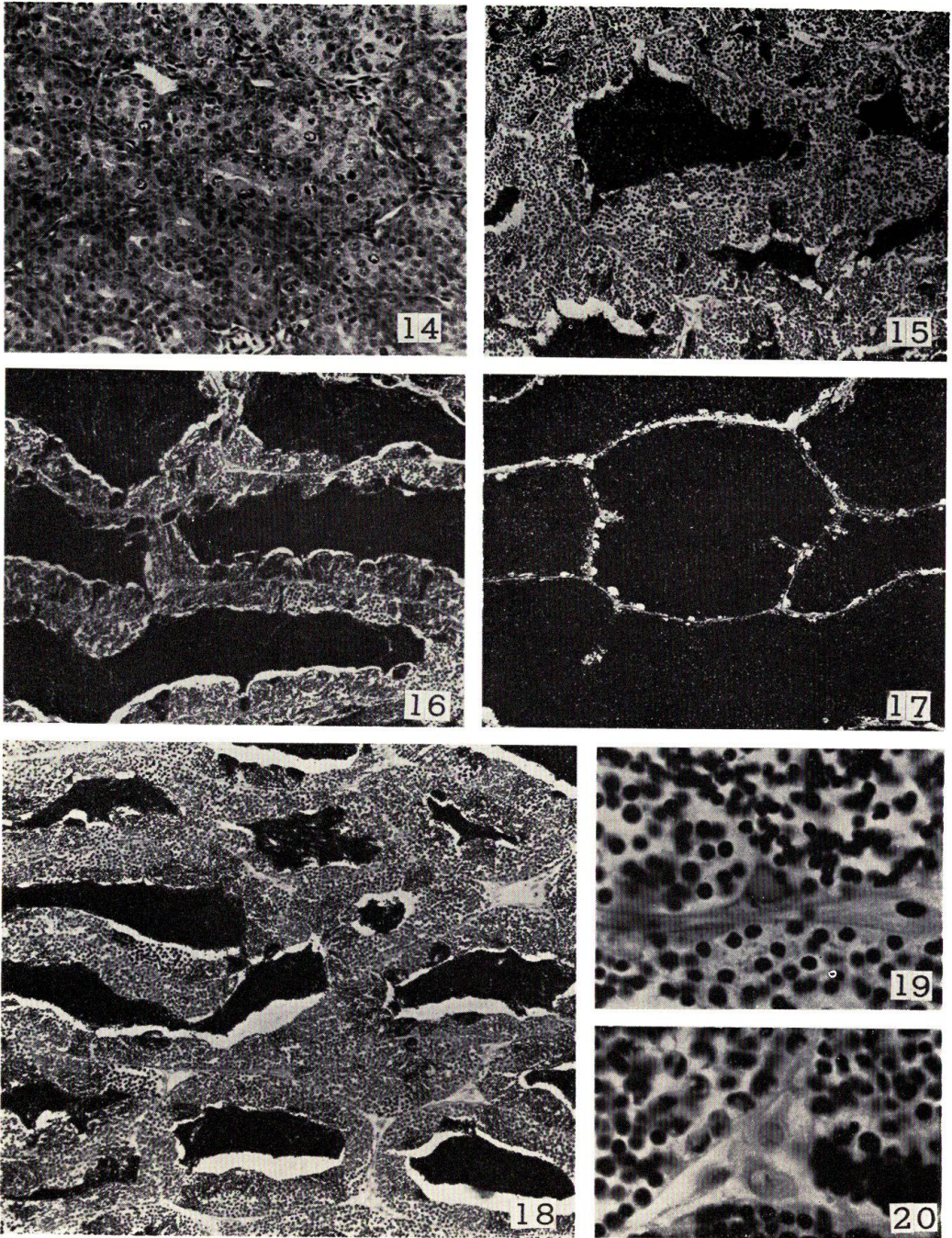
Fig. 1. A spermatogonium present independently in the testicular lobule: Fig. 2. A cluster of spermatogonia: Fig. 3. A spermatogonium in mitotic metaphase: Fig. 4. A cluster of spermatogonia in mitotic metaphase

Figs. 5-13. Sections through testes of maturing fish captured on September 27 in the coastal sea off Atsunai (group II). Magnification, $\times 1000$

Fig. 5. A cyst of spermatogonia: Figs. 6 and 7. Primary spermatocytes in the post-synaptic stage: Fig. 8. Primary spermatocytes in the first maturation division: Fig. 9. Secondary spermatocytes: Fig. 10. Secondary spermatocytes in the second maturation division: Figs. 11 and 12. Spermatids in spermiogenesis: Fig. 13. Mature spermatozoa



O. HIROI & K. YAMAMOTO: Changes in the testis of the chum salmon



O. HIROI & K. YAMAMOTO: Changes in the testis of the chum salmon

PLATE II

Fig. 14. Testis of an immature fish captured on April 20 in the northern Pacific (group I). Note the spermatogonia as an exclusive germinal element in each testicular lobule. $\times 200$

Fig. 15. Testis of a fish caught on September 27 in the coastal sea off Atsunai (group II). Testicular lobules contain masses of spermatozoa in the lumen and germ cell cysts in various stages of spermatogenesis in the periphery. $\times 100$

Fig. 16. Testis of a fish fixed on September 29 at Chiyoda (group V). Spermatozoa fairly increase in amount in each lobe. Germ cell cyst in various maturational stages are still noticed, while spermatogonium no more exists in the lobules. $\times 100$

Fig. 17. Testis of a mature fish collected on October 16 at Chiyoda (group VI). Testicular lobules are completely packed with masses of mature spermatozoa. All the germ cell cysts have already disappeared from the lobules as a result of full maturation of germ cell components into spermatozoa. Compare with Figs. 15 and 16. $\times 100$

Fig. 18. Testis of a maturing fish captured on September 28 at Ōtsu (group IV), showing the fusion of the testicular lobules. $\times 100$

Fig. 19. Testis of a maturing fish of group IV. Note that the somatic cells in the lobule contain distinct cytoplasmic vacuoles. $\times 1000$

Fig. 20. Testis of a maturing fish of group IV, indicating interstitial cells in the interlobular space. $\times 1000$