The morphology of maturing and matured testes of the sockeye salmon (Oncorhynchus nerka); with a comparison to the testes of the rainbow trout (Salmo gairdnerii)

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The University of Montana
BY THE STUDY OF THE ECOLOGY OF THE THREE-SPOKED SQUILL (Corymorphus nortii); WITH A
COMPARISON TO THE ECOLOGY OF THE HAMLETT SQUILL
(Salpa minimorii)

by

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Montana State University

Approved:

[Signature]

Chairman of Board of Examiners

[Signature]

Chairman of Committee on Graduate Study
THE MORPHOLOGY OF MATURING AND MATURED TESTES OF THE
Sockeye Salmon (Oncorhynchus nerka); WITH A
COMPARISON TO THE TESTES OF THE RAINBOW TROUT
(Salmo gairdneri) *

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* This investigation was done under the general direction of
Dr. L. C. Brown to whom the writer is indebted for many valuable
suggestions. The writer is also grateful to Mrs. C. B. Castle and
P. L. Wright for their aid.
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Introduction

Most investigators working on the gonads of teleosts have been concerned with the recognition of germ cells in the embryo and following their lineage in an attempt to answer theoretical questions bearing on the early segregation of these cells. The studies that have been made of the morphology and germ cells of the adult testes have shown that there is a diversity among many of the species studied and that study of other species was pertinent.

For this study, the sockeye salmon (Oncorhynchus nerka) was chosen (Plate 1). The Pacific salmon of the genus Oncorhynchus holds a unique position among the teleosts. Upon reaching sexual maturity, the males have an extreme development of the secondary sexual characters. There appears a pronounced hump on their backs, their jaws become elongated and distorted, and the skin becomes thickened, embedding the scales (Pl. 3). Both the males and the females die soon after their single spawning season.

As a basis for comparison, testes from rainbow trout in approximately the same stages of development as the salmon series were obtained. The rainbow (Salmo gairdneri) is classified in the family Salmonidae with the Pacific salmon, but it does not develop secondary sex characters to a high degree and generally spawns more than once in its life span. Another point of difference between them is
that the rainbow spawns in the spring whereas the salmon
used in this study spawned in the late fall and early winter.

In addition to a study of the testes, a study was
made of the changes occurring in the testicular ducts.

Review of the Literature

(a) The germ cells: Russbein ('30) in working with
the brown trout found no evidence of any epithelial cells
being transformed into germ cells. He suggested the theory
that germ cells arc segregate early in the development of
the embryo rather than being derived from somatic cells.
Further studies on teleosts have verified that there is an
early segregation of the so-called "primordial germ cells".
These primordial germ cells have their origin in the endo-
derm, and by migration or by forces of growth they reach
the site of the germ-gland anlagen (Jarvis, '30; Dodds, '19;
Richards and Thompson, '21; Lann, '27). Allen ('11) found
the same to be true for the gonoids urina and gonidiosaurus.

Studies of the higher vertebrates have shown that the
primordial germ cells do not necessarily become the functional
germ cells in the adult gonad. Birket ('29) observed two
generations of germ cells existin in the rat. These he
could distinguish from each other because the cells of the
second generation, the secondary germ cells, do not arise
until the primary germ cells have disappeared. These second-
ary germ cells he found to arise from the epithelial cells
of the gonad. In the chick he could not separate the first
and second generations of germ cells as they were present together. Wernby (16) followed the transition of peritoneal cells into germ cells in \textit{Rana temporaria}, but Burger (17) found that the primordial germ cells give rise to all future germ cells in the urodele \textit{Elephantopus}.

Most workers dealing with the cyclostomes and teleosts find that the primordial germ cells give rise to the functional germ cells. Working with the brook lamprey, Ekelberg (21) traced the definitive germ cells to their origin from primordial germ cells. Dohi (24) followed the primary germ cells in the gonads of the European salmon to the time of sex differentiation, and he believed that the functional sex cells arise as their linage descendants. Beckmann (14) and Kann (27) could find no transition of somatic cells into germ cells in \textit{Amia canadensis} and \textit{Cottus baileyi} respectively. Richards and Thompson (21) studied the primordial germ cells from the time of their first recognizable appearance in \textit{Fundulus} embryos to the twenty-fourth day. They felt that there could be no doubt in the identity of these germ cells because of their distinctive characteristics. \textit{Essenborg} (23), however, found a different picture in the viviparous teleost \textit{Ziphophorus belleri}. In the females, all the primordial germ cells disintegrate, and the definitive germ cells come from peritoneal cells. In the male, the fate of the primordial germ cells is less certain, but definitive germ cells originate from peritoneal cells.
As to the source of germ cells for each successive spawning period, Turner ('19) believed that in the perch, after each breeding season, germ cells migrate from a cord outside the testes into the testes and divide to form the cysts of germ cells. Foley ('23) suggested that this same condition exists in Umbra, cottus (Hann, '27), Kundulus (Matthews, '30), and Hoximus (Fullough, '32) always have resting germ cells along the sides of the lobules acting as a reserve supply from which the sperm of the next breeding season are formed. There is also the possibility that germ cells can be seasonally formed from peritoneal cells like the definitive germ cells are first formed in Xiphophora.

(b) Structure of the testes: the testes of teleosts can be divided into two types, acinus and radial, according to their method of development. Hann ('27) made a thorough study of the embryology and development of the radial type testes in Cottus Lairdii. The primordial germ cells divide and form nests of cells among the stroma. A sperm duct appears as a mesodit in the dorsal stroma portion of the testes. The stroma cells lining the duct later form a distinct epithelial lining. This main sperm duct and the blood vessels in the 59-day-old fish lie near the mesorchium. Secondary ducts lead off from the main duct as excavations in the stroma. The secondary ducts may branch several times before they reach a germ cell nest. Any of them do not
become lumminated with cysts of germ cells until the second year. By the time *Cottus* is 1½ days old there is a dorso-medial hilus of connective tissue containing the primary duct and blood vessels. At about 12) days of age, some of the secondary ducts have become lumminated with the sperm cell cysts changing them into tubules. Development from this stage on consists of an increase in the size and complexity of the tubules.

The testes of the two Cyprinidae *Carassius* and *Thorinus* are essentially similar to *Cottus*. Stromaten ('31) described the testes of the gold fish at the time of sex differentiation as consisting of nests of germ cells separated into lobules by connective tissue. In older embryos the spermatocytes become arranged around a central lumen to form seminiferous tubules. Stromaten did not describe any action played by secondary ducts in forming connective tubules between the seminiferous tubules of germ cells and the main sperm duct. Allough ('32) studied only the nature testes of the minnow *Thorinus*. Their testes show no definite structure, but the lobules tend to run from the ventral side to a hilus on the dorsal side of the testes.

The testes of *Millepora* belong to the acinus type, in which the seminiferous tubule formation is greatly modified (Aschenberg, '23). The early testes are similar to those of *Cottus* with nests of primary germ cells segregated by strands of connective tissue. Further differentiation
sees a proliferation of the peritoneal cells segregating
the primary germ cells to the periphery of the gonad. The
epithelial cells first form a solid sex cord parallel with
the long axis of the testis in its center. This cord of
cells acquires a lumen and sends off branches which in
turn become subdivided. The main tube becomes the sperm
duct. The epithelial cells lining the tubules gradually
assume a spherical shape and staining properties similar
to the primary germ cells. An increase in the size of
these secondary germ cells obliterates the lumen of the
acinus, and each testis is comparable to a bunch of
grapes with the main sperm duct as the stem. The primary
germ cells are segregated and never act as the definitive
germ cells. Van Wort ("25) described the mature testes
of Xiphophorus. The testes are fused, but the sperm duct
remains double until it fuses at the posterior end of the
testes. The more matured cysts of germ cells are toward
the center of the testes and the immature ones in the
periphery. Each cyst of germ cells is enclosed in a thin
membrane.

Lobistes and Ctenodus are viviparous poeciliids like
Xiphophorus. As far as the author knows, the embryological
development of their testes has not been worked out, so it
is impossible to state whether they have the same develop-
ment as Xiphophorus. The mature testes of Lobistes, accord-
ing to Vaupel's ("29) description, are similar to the testes
of Xiphophorus. Eldine ('36) considered the nodular region in Lebesitea to be possibly homologous to the nodular development in the testes of higher vertebrates. The testes of Carbusia have a central testicular duct but no prominent nodular region, and the cysts are packed in diffuse strands of connective tissue rather than having the more tubular arrangement of the testes like the other two poccillids (Geiser, '24).

With the development of viviparity and the consequent necessity for longer separate existence of sperm, the poccillids seem to have developed modifications for the care of the sperm which are not found in oviparous fish. The sperm are retained within the cysts until they are completely neotomorphosed, arranging themselves in a ring with their heads against the cyst walls. The sperm are thus in a form of spermatozooeae, which individuality they keep until they are discharged from the genital aperture (Geiser, '24; Van Bors, '25; Vampol, '29). Vampol ('29) found that in Lebesitea the cells of the cyst wall act as Sertoli cells and that these cells become noticeably enlarged shortly after the sperm shed their cytoplasm. Presumably the Sertoli cells ingest the shed cytoplasm.

Lundulus and the Ibera both have fused testes and a nodular region of connective tissues containing the main sperm duct and the blood vessels. Matthews ('38) described the testes of Lundulus as having a tubular arrangement
inasmuch as the lobules from the main sperm duct do not ramify. The testes of *Fundulus* more closely resemble the pocillids than does the perch. Turner ('19) found that on the ventral side of the perch testes there is a deeply embedded connective tissue core from which radiate septa. The septa join the testes wall and so divide the organ into irregularly shaped lobules. The lobules (tubules of Hann) may be twice bifurcated before they reach the testes wall. At the commencement of maturation, the lobules become divided into cysts of germ cells imperfectly separated by connective tissue cells. There does not seem to be any definite specification of maturation from the center to the periphery of the lobule such as found in the pocillids.

Materials and Methods

The nature sockeye salmon, *Oncorhynchus nerka*, used in this study were the land-locked salmon found in Flathead Lake, Montana.* On October 2, prior to the spawning period, adult salmon were caught by trolling. Those ready to spawn were obtained during the seining operations of the State Hatchery at Somers on November 15. Salmon taken by means of a drag hook on January 4 were believed to have finished spawning. All of the January fish were in various stages of decay, and several males showing only slight signs of life.

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* The October salmon material was procured by Dr. E. B. Castle of Montana State University and kindly loaned to the author. The author also wishes to acknowledge with gratitude the material furnished him by the Federal Fish and Wildlife Service and by the Montana Fish and Game Department.
were picked up on the shoreline. Age determinations made from the scales and otoliths showed the mature fish taken in November and January to be in their fourth year.

The rainbow trout were procured from the Federal Hatchery at Creston on the Flathead River, Montana. Testes of the trout, to be compared to the testes of mature salmon, were from three- and four-year-old males taken from the stock pools on March 6 and April 13. On May 10 trout which had been stripped a month previously were obtained.

Immature salmon and trout were taken from the stock pools of the State Hatchery on Flathead Lake at Polson, Montana on December 3, January 4, and March 6. The salmon were from spawn of the lake sockeye and were three years of age. The rainbow trout were two years old.

The material for this investigation was immediately weighed and measured in the fresh condition, excepting for the testes, which were weighed after fixation. The gonads and their ducts were removed in the field and placed in either Bouin's picric-formol, copper's fluid, or Boucher's mixture of alcohol-formalin-acetic acid. To insure fixation, the larger, more matured gonads were cut into small portions.

The material obtained by Dr. Castle had been treated in a similar manner, excepting that the tissues had remained in Bouin's fixative from 1936 to 1941.

Tissues were embedded in paraffin and cut from 5 to 6 thick. Iron hematoxylin destained with a saturated solution
of picric acid was used to stain most of the tissues. A counterstain of eosin was used in some cases. Sections stained in Harris's acid haematoxylin counterstained with eosin, and also Mallory's triple stain proved valuable for study.

The small immature testes were cut serially either in cross- or longitudinal-sections. However, it was impractical to make serial sections of the whole gland of the nearly mature or matured testes due to their length and large size, so cross-sections were cut as near as possible to the mid-region of the testes proper. The portions taken were approximately a quarter of the cross-section and were cut so as to include the main blood vessels and the sperm duct. Sections of the sperm duct, posterior to the testes, were taken half way between the point where the duct leaves the testes to where it enters the uregenital sinus. Longitudinal-sections as well as single cross-sections from various regions of the testes and ducts were also made.

Observations

I. Immature Testes

(a) Salmon: The testes of the three-year-old salmon are small paired elongate bodies, each suspended by a mesorchium from the ventral anterior portion of the air bladder (Fig. 4). In cross-section the largest portion of
the testes from four fish measured on the average .84 m. at their maximum dorso-ventral width and .72 m. at their maximum lateral width. Some are triangular while others are oval in cross-section. A thin transparent peritoneal membrane, the tunica vaginalis of mammals, covers the testes. A thread-like sperm duct continues from their posterior end to the urogenital sinus.

The mesorchium arises from the dorsal margin of the gonad. The genital vein and artery, embedded in the dorsal peripheral stroma of the testes, are situated at the base of the mesorchium. Numerous branches are given off by these blood vessels, and the testes are well provided with capillaries which traverse through the strands of testicular stroma. Dorsal to the blood vessels there is a space of variable size. The space contains a liquid, probably lymphatic, which takes a light brown stain in iron hematoxylin preparations and a light pink color when stained with hematoxylin-cosin. An occasional lymphocyte may be seen in the fluid of the space. It has no connection with the testicular canal and is interpreted as a space left by the incomplete fusion of the coelomic walls when the canal pushed ventrally into the coelom.

The sperm duct is seen as a longitudinal slit in the stroma near the mesorchium. The term "testicular duct" will be used here to designate that portion of the sperm duct which is incorporated within the testicular tissue, and
"extra-testicular duct" to designate that portion of the sperm duct which is its continuation from the posterior end of the testes. At the anterior end of the testes no testicular duct is formed. It starts at about the anterior third of the organ as a small slit-like lumen and enlarges as it progresses posteriorly. The cells lining the sinus are loose connective tissue cells of the sperm. Toward the posterior end a more distinct epithelial layer is formed, and the extra-testicular duct in some cases has a poorly defined cuboidal epithelium lining its narrow lumen. A few spermatogonia can be seen along the walls of the testicular duct. The lumen of the testicular duct is variable in shape and sends out short branches through the connective tissue of the testis to the more proximal nests of germ cells.

The germ cells of the immature testes are spermatogonia in nests surrounded by folliculo cells, and the nests in turn are separated into lobules by fine partitions of connective tissue. The lobules vary in size, shape, and the direction in which they run. There appears, however, to be a general tendency for them to run in a dorso-ventral direction.

The spermatogonia are by far the largest cells to be found in the immature testes (Fig. 5). The average diameter of 150 of these cells, selected at random from the testes of three different salmon, was 0.44. They ranged in diameter from 12.4 to 6.8. The spermatogonia are spherical cells
with typically one large circular nucleolus, but there may be as many as three. In the clear nucleolus there is a system of delicate radiating linin fibrils which apparently have their origin from the nucleolus. The chromatin granules are scattered along the linin network. The cytoplasm of the spermatogonia is clear with slight granulation. The nuclear membrane is well defined, but the cell membrane is not. In hematoxylin-eosin preparations the cytoplasm takes a light pink stain, the chromatin a deep blue, and the nucleoli a deep blue with a pink border. In iron-hematoxylin the cytoplasm has a faint grey granular tinge, the chromatin stains black, and the nucleoli a yellow-brown. The spermatogonia can be readily identified from the other cells in the testes by their large size and their comparatively clear cytoplasm and nucleolus.

Counts of spermatogonia undergoing mitosis were taken along linear distances from one side of the testes to the other by using a mechanical stage and a ruled ocular. There was an average of 0.4 cells per 1,000 in some active stage of mitosis; 3,000 cells were counted in three different salmon testes. There is no sign of degeneration among any of the germ cells. In cross-sections of the testes there are from one to twenty-five spermatogonia in a nest. The largest germ cells are those found in groups of only two or three. As the cells increase in number, presumably by mitosis and not by conversion from epithelial cells, the nests become
larger and the germ cells slightly smaller. In the immature salmon studied there are no epithelial cells intermediate between somatic cells and germ cells which might suggest a transformation into germ cells. It is possible that such a transformation might take place in younger stages than those examined.

Surrounding the nests of spermatogonia are follicle cells. These cells are variably shaped to fit the curvature of the nests. The nuclei are approximately one-third the size of the germ-cell nuclei and appear darker due to the more condensed chromatin granules. They have no conspicuous nucleolus. No stages of mitosis were noticed among the follicle cells of the immature salmon. Around the smaller nests, the follicle cell nuclei are in close proximity to each other. Around the larger nests, the nuclei are further apart, and the cells are stretched into a more spindle-like form. The nests of germ cells are generally circular but may vary much in shape. Some of them appear as short cords of germ cells bearing a superficial resemblance to tubules.

The larger nests of germ cells, which occur more often in the testes procured in March than in those obtained earlier in December and January, have a small lumen in their center. When traced in serial sections, most of these lumina are seen to end blindly, but near the necrochorium the lumina of some of the cords of germ cells have become continuous with secondary ducts, converting them into short tubules lined
with spermatogonia and emptying into the main testicular duct (fig. 6).

Nesting in between the germ cell nests are long narrow strands of connective tissue. They have elongated nuclei which stain much darker than any of the other nuclei in the testes with the exception of the blood cells. Coursing through this strum are numerous capillaries carrying blood to all parts of the testes.

Longitudinal-sections made of the immature testes show the structure of the nests and the relationship of the strong tissue to be exactly the same as they appear in cross-section. There is no more tendency for the nest bundles to run in a longitudinal direction than laterally. The strands of connective tissue forming the lobules have a tendency to run in a ventral-dorsal direction as they do in cross-section.

(b) Trout: Although the immature salmon material was three years old and the immature trout material two years old, two of the trout testes were approximately in the same degree of development as the salmon. The other two trout examined had their testes slightly further developed. The average for the cross-sections in the mid region of the two year old trout testes was larger than the average for the three year old salmon testes, being .47 mm. in maximum dorsal-ventral width and 1.16 mm. in maximum lateral width. The immature trout and salmon testes are basically similar in all their structural details. The average diameter of one hundred
spontaneous from the testes of three trout measure 7.7 fr, which is somewhat smaller than in the salmon. The two more mature trout testes have larger cysts with more frequent lumination. The testicular ducts of these two testes show more complexly branched secondary ducts leading from them, and the space above the blood vessels is reduced as compared to the two less mature trout testes and the salmon testes.

(c) Discussion: From the structure of these immature testes, it would appear that the trout and salmon testes have a development similar to Mann's ('27) description of the mechanics of the radial type development in Cottus bairdii. The blood vessels and the main testicular duct are situated in a dorsal hilus as in Cottus, and, from the testicular duct, secondary ducts are given off through the stroma cells. Some of the secondary ducts are branched, and a few of their lumina have reached nests of germ cells to incorporate them in a system of tutes.

Primordial germ cells and spermatogonia from different species of fish have similar distinguishable characteristics. The appearance of the spermatogonia in the immature trout and salmon are similar to the descriptions given by Prey ('26) for spermatogonia from L happiest by Coicor ('24) for spermatogonia in Centmuia. A table of the nuclear diameters of primordial germ cells found in different species of fish may be of interest here for comparison (table 1). Although there is perhaps a change in the size of the nucleus from the primordial

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### Table I

Comparative Diameters of Germ Cells

| Material               | Investigator          | Nuclear Diameter of
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<thead>
<tr>
<th></th>
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<th>Trichorial Germ Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fundulus</td>
<td>Richards and</td>
<td>7.2, average</td>
</tr>
<tr>
<td></td>
<td>Thompson, '21</td>
<td></td>
</tr>
<tr>
<td>Ichthysus</td>
<td>Eunsonberg, '23</td>
<td>8.4 - 14.4</td>
</tr>
<tr>
<td>Ichthysus</td>
<td>Biddie, '36</td>
<td>6.3 - 6.5</td>
</tr>
<tr>
<td>Incanthus perle</td>
<td>spermatogonia</td>
<td>6.3 - 12.4</td>
</tr>
<tr>
<td>Palmo maireri1</td>
<td>spermatogonia</td>
<td>5.6 - 12.4</td>
</tr>
</tbody>
</table>

Germ cell stage to the spermatogonia, the similarity in size is noticeable.

II. Testes in Maturation Stages

(a) Salmon: The two salmon caught on October 2, 1938, did not yet have fully developed secondary sexual characteristics. The length of the upper jaw averaged one centimeter less than the average for fifty completely matured salmon caught in the same year (Table I). It might be noted here that the 1/38 salmon were somewhat larger than the salmon caught in 1941. The average weight of fifty male salmon caught in November, 1941, was 277 grams less than fifty males weighed by Dr. Castle in November, 1938.
### Table II

<table>
<thead>
<tr>
<th>Material Studied</th>
<th>Number of fish</th>
<th>Sex</th>
<th>Average length in centimeters</th>
<th>Average weight in grams</th>
<th>Average length of jaw in centimeters</th>
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<td>Immature 3-year salmon: Dec. 3, '42; Jan. 4 and Mar. 8, '42.</td>
<td>7</td>
<td>male</td>
<td>12.5</td>
<td>37.3</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>female</td>
<td>12.7</td>
<td>37.2</td>
<td>0.6</td>
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<tr>
<td>Salmon in maturation stages Oct. 2, '38.</td>
<td>2</td>
<td>male</td>
<td>31.1</td>
<td>737.1</td>
<td>2.7</td>
</tr>
<tr>
<td>Spawning salmon: Nov. 15, '41.</td>
<td>59</td>
<td>male</td>
<td>27.0</td>
<td>540.3</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>female</td>
<td>24.9</td>
<td>44.5</td>
<td>1.7</td>
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<tr>
<td>Post-spawning salmon: Jan. 4, '42.</td>
<td>6</td>
<td>male</td>
<td>25.5</td>
<td>435.5</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>female</td>
<td>27.3</td>
<td>389.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Immature 2-year trout: Dec. 3, '41; Jan. 4 and Mar. 8, '42.</td>
<td>4</td>
<td>male</td>
<td>12.9</td>
<td>49.4</td>
<td></td>
</tr>
<tr>
<td>Spawning trout: Mar. 8 and April 15, '42.</td>
<td>6</td>
<td>male</td>
<td>21.0</td>
<td>274.5</td>
<td></td>
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<tr>
<td>Post-spawning trout: May 16, '42.</td>
<td>6</td>
<td>male</td>
<td>19.7</td>
<td>197.3</td>
<td></td>
</tr>
</tbody>
</table>

*Measurements of length made from posterior extremity of pterotic bone to the end of the scales on the tail; taken with a cloth tape.

**Length of the upper jaw from the middle of the external nares to the tip of the snout; taken with calipers.
The salmon testes in various stages of maturation have a complex structure. The lobules are very intricate but run most generally from the periphery toward a dorso-medial testicular duct. The walls of the lobules are lined with large cysts of germ cells so the testes appear to be made up of irregular chains of cysts. The lobule cysts are thin strands of connective tissue which are difficult to trace. In the ripe salmon testes, which have well defined lobule lumina filled with dark staining masses of sperm, the general ground structure of the testes is more readily understood.

The October salmon have the germ cells in various stages of spermatogenesis (Fig. 7). Each cyst contains cells which are in the same stage of maturation. All of the cells within one cyst right to in the metaphase of the first maturation division, whereas the germ cells in a cyst next to it right all be spermatids. There is no segregation of spermatogenesis from the cortex to the reducta of the testes. The cysts are enclosed in an extremely thin membrane of follicle cells which usually breaks in the spermatid stage. Only close observation detects the presence of these cyst walls. The cysts are packed with germ cells and have no lumen. Most of the lobules have a lumen of variable size in which are spermatids or spermatocytes. It is the breaking of the ripe cysts (to release their germ cells) that forms and enlarges the lumen of the lobules. The few lobules without lumen are fully packed with unbroken cysts of germ cells.
Along the walls of the lobules, and not infrequently embedded in their tissue, are occasional normal resting spermatogonia which have not formed cysts. They have exactly the same appearance as the spermatogonia in the immature testes. The average nuclear diameter of 150 cells measured in three different testes was 7.5 μ, a slight increase compared to the spermatogonia in immature testes, which might indicate a growth period previous to active multiplication.

It is difficult to distinguish between the primary spermatocytes and the later generations of the spermatogonia as they both have a nuclear diameter of about 6.6 μ. The material at hand shows no nucleoli in the primary spermatocytes. The preponderance of the cysts contain primary spermatocytes undergoing a nuclear condensation into an apparent syngonon stage. Primary spermatocytes in other cysts have the greater portion of their chromatin material drawn to one pole so as to form a typical bouquet stage (.i.e. 7). No certain post synaptic spiretes were observed. Later stages show the nuclear membrane to disappear, the small, mostly oval chromosomes to line up in the metaphase with definite spindle fibers formed, and the first maturation division completed.

The secondary spermatocytes have a nuclear diameter of 4.5 μ or are one-third the nuclear size of the primary spermatocytes. Few resting secondary spermatocytes are present, therefore the second maturation division probably takes place rapidly.
The spermatids have a nuclear diameter of 3.4 μ, showing a reduction in size from the secondary spermatocytes. It is in the spermatid stage that the germ cells generally become free of their investing membrane and lie in the lumen of the lobule. Since the preparations were not made for cytological detail, no attempt was made to trace the nuclear and cytoplasmic metamorphosis from the spermatid to the mature spermatozoa. There is, however, an obvious condensation of the spermatid nuclear material into a homogenous dark staining sphere.

The mature spermatozoa has an oval-shaped head, narrowest at its anterior end. The head stains an intense black in iron-hematoxylin preparations. The widest diameter of the head is approximately 1.1 μ and the tail piece three to four times this length. The sperm move to the center of the lobule's lumen where they become arranged so that their tails are to the center of the sperm mass. The masses of sperm are irregular in size and shape, showing no tendency toward the formation of spermatozoa. There are no serolli cells. The sperm remain free in the lobule lumina unattached to any cells.

The testicular duct contains a mass of sperm and also some liquid which stains a faint brown in iron-hematoxylin and a light pink in hematoxylin-cosin. The epithelial cells of the duct are mostly columnar, but in places they are cuboidal. The nuclei of these cells are nearly all centrally located.
(b) Discussion: The material indicates that the secondary testicular ducts of the immature testes have become continuous with the lumina of the germ cell nests so that the germ cells come to line the walls of lobules, the lobules being comparable to lobate tubules connected with the main sperm duct. There are probably also communications made between the lumina of various germ cell nests, although none of the stages at hand show this actual procedure. The nests of germ cells seen in the immature testes are therefore not homologous to the cysts of germ cells present in the maturing testes. That probably takes place is that each of the primary spermatagonia lining the tubule walls undergoes successive division to form cysts of germ cells enclosed in a delicate membrane of follicle cells. A few spermatagonia, however, remain in a resting condition and do not form cysts. The formation of cysts and the multiplication of the cells within the cysts increases the size of the testes and packs the lobules so that there are no lumina. In the later maturation stages the spermatids and spermatocytes break free of the cysts so that the lobules acquire a patent lumen at this time. The lumen of the lobules communicate directly or indirectly with the main testicular duct.

The general pattern of spermatogenesis in the salmon follows that of Salmo (Hanna, '27) and the perch (Romer, '19).
Unlike in viviparous fish, the salmon spermatozoa do not form spermatoaphore and are not retained within cysts but migrate into the lobule lumina or into the sperm duct.

III. Ripe testes

(a) Salmon: The mature male sockeye salmon from Flathead Lake (Fig. 3) develop secondary sexual characteristics similar to the ocean running pink salmon (Oncorhynchus gorbuscha) as described by Davidson and Wootton ('36).

Table II shows the increase in jaw length of the male as compared to the female, which undergoes only a slight change. The age of the anadromous sockeye, when sexually mature, varies from three to eight years (Gilbert, '22; Gilbert and Rich, '27). Scales and otoliths were examined from fifty salmon of both sexes taken in the spawning run of November, 1941, at Sockeyes. All of these fish were found to possess three annuli on their scales and otoliths which would indicate they were four years of age.

The paired ripe testes of the salmon are so enlarged as to take up more coelomic space than the rest of the viscera. They are white finger-like organs with their rounded anterior ends against the anterior wall of the coelom. Posteriorly, they are constricted into a short duct which leads to the urogenital sinuses. The lobules and the ducts are distended with a liquid mass of milt.

The relationship of the mesorchium has changed in the mature testes as compared with the immature testes. The
immature testes have the mesorchium suspending the testes from their dorsal end, the testicular duct and blood vessels also being dorsally located. The ripe testes have the mesorchium attached to their medial surface, and the testicular duct and blood vessels are located on the dorso-medial side of the testes. The point of attachment of the mesorchium is on the medial side, approximately one-third the distance ventral from the dorsal point of the testes. From its principal point of attachment, the mesorchium is incompletely fused along the dorso-medial surface of the testes. The change in position of the mesorchium is probably brought about by the growth of the testes, which crowd against the stomach and liver, located ventral and medial to them. This crowding results in a rotation of the testes away from these organs, carrying the once dorsally located hilus to a dorso-medial position. A similar rotation is not seen in the posterior continuation of the testes, the extra-testicular duct. The point of attachment of the mesorchium remains on the dorsal side of the duct.

The spermatic vein and artery enter the testis near its anterior end by way of the mesorchium. These blood vessels were traced anteriorly in one specimen. The spermatic vein was found to have its origin from the posterior cardinal vein near to its point of entry into the suverian duct. The spermatic artery had its origin from the dorsal aorta. The vein and artery, embedded in the peripheral dorso-medial
stroma near the main spermatid duct, together continue posteriorly in the testes. Their position is variable in the extra-testicular duct, sometimes running within the duct and sometimes in the mesorchium outside of the duct. Near the posterior end of the extra-testicular duct the blood vessels are too small to be traced macroscopically. Sections of the cloacal region show the spermatic blood vessels to have a diameter capable of containing no more than ten to twenty blood cells. The vein and artery give off numerous branches through the connective tissue, and capillaries are found throughout the testicular stroma. Frequently present in the hilus of testes are lymph spaces, so situated as to possibly represent vestiges of the dorsal space described for the immature testes. A few of the smaller lymph vessels are present in the connective tissue of the lobule septa.

The testicular duct is irregular in shape, secondary ducts opening into its proximal side from all angles (Fig. 1). It increases in size as it progresses posteriorly to where it merges with the extra-testicular duct. The duct is full of a free mass of sperm. The epithelial lining of the testicular duct is irregularly cuboidal to columnar. The cytoplasm of the epithelial cells takes a basic stain, making it difficult to differentiate between the nucleus and the cytoplasmic portion of the cell. The cytoplasm of the stroma does not take this basic stain. The normal epithelial nuclei
are oval to spindle shaped and are situated usually in about the center of the cell. Most cells of the duct of the liver have a large vacuole located between the nucleus and the limb of the cell. These vacuoles are clear, with no recognizable content. Any of the vacuoles are so large they exert the retired into a cistern-shaped. This vacuolization does not appear in the convoluted tissue, cells immediately below the bile-dense and elsewhere in the technical stroma. More explicit account of this vacuolization is given in the description of similar occurrence in the extra-cellular in the technical stroma.

The latices in the side isthmus are of greater size and are arranged as to form a structure. Usually, they run in a toothed to dental direction to a point in the isthmus, and in the largest end, usually end up to remain in this base. The actual reproduction of a portion of a tooth is made by the application of the lower isthmus. The ends of the isthmus are minute cavities of convoluted tissue near the isthmus, the central portion to the convoluted tissue.

Some isthmus stains may vary the size and location of these to be present, but not in the teeth. The latices of the isthmus are filled with more oriented so that their ends are toward the center of the teeth. In the case of convoluted tissue, there is no indication of any 21 cells.
Fig. 1. Serial reconstruction of a portion of a fully matured salmon testis. Figures drawn with camera lucida.

10. Each section is cut 3 μ. The lobules showing connection with the main sperm duct are filled in solidly. bl. v., blood vessel; l.l., lumen of lobule; l.s., connective tissue of lobule septa; t.d., testicular duct.
The cysts of germ cells undergoing maturation have disappeared in the ripe testes, but irregularly placed along the lobule walls or embedded in the stroma are occasional resting spermatagonia (Fig. 1). They are present singly or in cysts of from two to ten, surrounded by follicle cells. The spermatagonia have clear cytoplasm and nucleoplasm, one or two large nucleoli, and reticulate chromatin. The average diameter of the spermatagonia was 9.2μ, approximately the same diameter as the spermatagonia in immature testes.

A majority of the spermatagonia have developed clear vacuoles like those found in the epithelial cells of the testicular duct (Fig. 1). In some of the testes this vacuolization is so frequent that it is difficult to find unaffected resting spermatagonia. Vacuoles also appear in the follicle cells but not in the connective tissue. Spermatagonia which are vacuolated lose their typical circular outline, and the nucleus assumes a sickle-form as though forced into that shape by the pressure of the vacuole. In some extreme cases the vacuoles have broken, releasing whatever product they may have into the lumen of the lobules. The nucleol of vacuolated spermatagonia lose their nucleoli and the chromatin becomes granular, taking a darker stain than the unaffected spermatagonia. The cytoplasm of the vacuolated cells takes a basic stain like the duct epithelium.

(b) Trout: The trout testes have essentially the same structure as the salmon testes. Testes from ripe trout have
the ducts and lobules filled with spermatozoa. Neither spermatoocyte nor spermatid stages are present. The numbers of resting spermatogonia present along the walls of the lobules vary considerably with the individual. In some, the resting spermatogonia literally line the lobule walls (Fig. 9), whereas, in others similar to the salmon, they appear at irregular intervals. The resting spermatogonia have the identical characteristics and size of the spermatogonia in the immature trout testes. None of the spermatogonia have the amoeboid shape indicative of migration.

A marked difference between the ripe salmon and trout testes is the complete absence of any sign of vacuolization or degeneration in any of the cells in the trout testes. None of the spermatogonia are vacuolated. The lining of the testicular duct, which is a cuboidal to columnar epithelium, has no indication of any vacuoles occurring in any of its cells.

(c) Discussion: The lobule structure of the matured salmon and trout testes is essentially similar to Turner's (1) description of the lobules in the perch testes, excepting that the salmon and trout lobules branch many times more. Although the dorso-medial hilus of the salmon and trout testes contains the central portion of connective tissue, there is no concentration of connective tissue which might be homologized to a connective tissue core such as is
found in the perch (Turner, '19) or in Helistes (Bildino, '36). Also there are no adipose bodies present in either the trout or salmon like Turner found in the perch connective tissue core. Turner thought that the abundance of elastic tissue, which he found to be present in the perch testes, served an important function in the expulsion of sperm. The elastic fibres in the testicular stroma and the ducts of the salmon and trout probably serve this function, as aside from the muscular contraction of the body wall, there is no special organ for ejaculation, and neither the testis' well nor the ducts are muscular.

The vacuolization of the spermatocysts in the ripe salmon testes is the first indication of the disintegration of these cells. The loss of good staining properties and definite cellular shape show the initiation of necrosis; this becomes more pronounced in the next stage. The trout testes in the spawning stage differ markedly from the salmon in that they exhibit no indications of vacuolated nor necrotic cells.

IV. Post-spawning Testes

(a) Salmon: All of the live salmon caught in January had finished spawning and were in various stages of decay. In the most extreme cases, the caudal and dorsal fins were completely rotted off, excepting for the base of the fin rays. Also the flesh along the dorsal ridge of the lump had turned yellow with decay. In less extreme cases only the
dorsal and caudal fins showed signs of rot. Many of the dead fish found washed up on the beach showed no more advanced stages of rot than actively swimming fish which were collected.

The testis' weight of ten salmon which had finished spawning averaged 0.4% of total body weight as compared to 0.7% average for testis from ten fish taken during the spawning run in November. The contraction of the testes after the expulsion of the greater mass of the sperms makes the septa of the lobules to appear a complicated maze. A few disintegrating sperm are present in the lobules and ducts of the post-spawning salmon. On examination of dead salmon also shows the presence of a few sperm remaining in the testes.

Cells lining the lobule walls are much more vacuolated than those in the ripe testes and exhibit unmistakable signs of pyknosis. In sections under low objective, the lobule septa look not unlike adipose tissue from which the fat had been dissolved. The cells of the stroma, as well as the spermatogonia and follicle cells, are vacuolated. The spermatogonia and follicle cells are the first affected with vacuolization as there is no evidence of vacuolization among stromal cells in the ripe testes.

One salmon caught in January had signs of rot only on the caudal fin. Testes from this fish show large vacuoles in the spermatogonia and follicle cells and smaller vacuoles
In the connective tissue cells (Fig. 11), the nuclei are shrunken with granular chromatin, taking a dark stain. The few sperm present in the testicular duct and lumina of the lobules appear normal.

Degenerative changes are more pronounced in five salmon which had their dorsal and caudal fins rotted off and their hump ridges decaying. It is impossible to stain the testicular tissue of these post-spawning salmon and get good differentiation. Vacuoles occur in nearly all the cells lining the lobule walls and are extensive throughout the stroma. Many of the vacuoles along the lobule walls have broken. The nuclei are markedly pycnotic, having different degrees of staining capacity depending on the amount of pycnosis which they have undergone. The most shrunken nuclei take the deepest staining due to more condensed cytoplasmic and nuclear material. Kernorrhexis is not evident. There is a liquefaction of many of the cells, so that some of the lumina contain a granular liquid with scattered nuclei and no cell walls distinguishable (Fig. 12). The liquid stains a light brown and the nuclei solid black in iron-hematoxylin preparations. The peritoneal cells of the membrane covering the testes and the cells of the mesorchium show only slight signs of necrosis. The epithelial cells of the testicular sperm duct are pycnotic, and the lumen of the duct contains a liquid with fragments of cells sloughed off from the epithelium. The blood vessels and
capillaries are full of erythrocytes, but these cells too are necrotic and in some cases have undergone liquefaction.

(b) Trout: the testes examined for the post-spawning trout were taken from fish on May 16. They had been stripped of their milt three weeks previously. These trout appeared to be normal in all respects.

The post-spawning testes are similar to the testes from spawning trout, excepting that the volume of sperm present is greatly reduced. The average testis weight of six post-spawning trout was 5.0% of the total body weight compared to 1.2% for six trout taken in March and April. The sperm which are present in the lobules and testicular duct do not show signs of disintegration. There are certainly no signs of vacuolization or of necrosis in any of the testicular cells (Fig. 13). The resting spermatogonia are typical with clear karyoplasm and a chromatin network radiating from one, two, or three large nucleoli. The average diameter of one hundred spermatogonia measured in three fish was 7.6μ, nearly the same as in the immature and spawning trout. There are occasional spermatogonia undergoing division, presumably in preparation for the sex products of the next spawning season. The larger spermatogonia are nearly always found singly and the smaller ones in cysts of two to eight cells. The spermatogonia are surrounded by smaller, irregularly shaped follicle cells which will form the fine membrane that encloses the large cysts of germ cells in maturation.
(c) Discussion: The source of the seasonal supply of germ cells in the rainbow trout is undoubtedly similar to the source in Phoxinus (Pullough, '39). In Phoxinus resting germ cells are present at all times of the year along the sides of the lobules. At the end of each breeding season, these germ cells divide and form cysts filling the lobules. In the salmon, however, the spermatogonia become vacuolated during the spawning period and then undergo pycnosis. In the authors' opinion this occurs due to approaching death. If this did not occur, the spermatogonia, remaining in a quiescent along the lobule walls, could fill the lobules with cysts of germ cells by successive division.

There is no indication that the spermatogonia present had migrated to their position along the lobule walls. Some of the spermatogonia have a ovoid shape as described for spermatogonia migrating into the efferent tubules (Turner, '1).

Near the termination of the life cycle of the male and female salmon there are exhibited degrees of decay in certain body regions before the fish dies. Even though some of their fins were completely rotted off and decay had set in on their backs, many of the salmon caught in January could still actively swim. The condition of first vacuolization and then pycnosis in the cells of the testes is merely one of these signs of approaching death. The trout, which normally do not die soon after their first spawning act, have a complete absence of this condition.
Inanition may be one explanation for the early appearance of degeneration among the spermatagonia in the otherwise apparently normal salmon at the time it is ready to spawn. The Pacific salmon practically cease to feed during the spawning run. They rely on stored fats for maintenance and the development of sexual products (McCone, '29; Gilbert, '32; Davidson and Shoeström, '36). Mason ('36) fed rats a standard casein diet which maintained their growth and health but caused the testes and spermatosoa to degenerate. Unlike the rat the spermatosoa in the salmon show no indications of disintegration until necrosis is well developed in other cells of the testes. This perhaps is due to the fact that the salmon spermatosoa are not dependent on Sertoli cells for nourishment as in the case of the rat. Turner ('37a) starved several hundred *O. kisutch* and found that there is a degeneration of primary spermatocytes and an inhibition of further formation of these.

It evidently is not an uncommon occurrence among fish for sperm to be present in the testes and the ducts after the spawning acts are completed, such is the case in the salmon and trout. Matthews ('38) found for *O. kisutch* that there are small clusters of sperm in the testes left over from the previous spawning period regardless of the time of year. Turner ('39) found that there is not complete expulsion of the sperm in the pouch and that scattered sperm can be found during the summer months after the spring spawning.
V. Extra-testicular Duct

(a) Salmon and Trout: In the posterior third of the coelomic cavity, each testis is constricted into a duct which leads to the urogenital sinus. Sections show that the constricted portion contains testicular tissue for a variable distance posteriorly. The testicular network is gradually reduced posteriorly but the duct seldom becomes a simple tube, even when the testicular network is completely absent. The irregularly shaped walls of the duct send projections into the lumen, and there are often sperm-conducting tubules within the duct itself. No germinal elements are found in the posterior portion of the duct. The paired ducts do not fuse until they enter the urogenital sinus.

The mesonephric duct arises dorsally and circles the posterior side of the air bladder. It enlarges into a urinary bladder which lies between the air bladder and the posterior portions of the sperm ducts. The urinary bladder is constricted at its posterior end where it enters the urogenital sinus just below the opening of the now partially fused sperm ducts. The urogenital sinus is short and opens almost directly to the exterior through a small papilla. The urogenital opening is just posterior to the anus.

The spermatic vein and artery are either embedded in the dorsal connective tissue of the duct or are in the
mesorchium. Capillaries and lymph spaces are numerous.

The lumen of the thread-like extra-testicular duct of the immature fish is very narrow and lined with cuboidal epithelium. No germ cells are found along the epithelium until it merges with the testicular portion of the duct.

The extra-testicular duct of the October salmon has a narrow lumen of variable shape (Fig. 14). Though there are sperm present in the lumina of the lobules and the testicular duct, there are no sperm present in the extra-testicular duct. The epithelium is mostly tall columnar but may be cuboidal in the same section. Vacuoles (2.3 to 6.8μ in diameter) occur toward the apex of the epithelial cells. The vacuoles have no recognizable content and take no stain. The nuclei of the epithelial cells are mostly basal but may be apically or centrally located.

The ducts of the November spawning salmon are distended with sperm (Fig. 15). The average diameter of the lumen is about twice the size of the lumen in the October salmon (see Table II for comparisons of lumen diameter, epithelial height, and vacuoles). The cells of the duct epithelium of the November salmon are cuboidal and show a marked decrease in height as compared to the October salmon. The vacuoles have increased in number and in size. The epithelium has the same appearance as the epithelial lining of the testicular duct in the same salmon. The cytoplasm takes a light basic stain in Alrich’s hematoxylin and in
<table>
<thead>
<tr>
<th>No. of fish</th>
<th>No. of fields</th>
<th>Total length of fields</th>
<th>Average % of vacuoles per nucleus</th>
<th>Average vacuole diameter*</th>
<th>Average epithelial height**</th>
<th>Approximate diameter of duct lumen**</th>
</tr>
</thead>
<tbody>
<tr>
<td>October salmon</td>
<td>1</td>
<td>50</td>
<td>2.27 mm.</td>
<td>34</td>
<td>4.0</td>
<td>20.4</td>
</tr>
<tr>
<td>in maturation</td>
<td>1</td>
<td>50</td>
<td>2.27 mm.</td>
<td>44% av.</td>
<td>3.0</td>
<td>13.2</td>
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<td>November spawning salmon</td>
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<td>50</td>
<td>2.27 mm.</td>
<td>86%</td>
<td>5.0</td>
<td>14.5</td>
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<td>2.27 mm.</td>
<td>92% av.</td>
<td>5.7</td>
<td>13.9</td>
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<td>January post-spawning salmon</td>
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<td>63%</td>
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<tr>
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<td>75% av.</td>
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<td>74% av.</td>
<td>7.3</td>
<td>12.2</td>
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<td>none</td>
<td>11.8</td>
<td>0.9</td>
<td>0.2</td>
</tr>
<tr>
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<td>50</td>
<td>none</td>
<td>10.0</td>
<td>1.3</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* The diameter of the first vacuole on the right side of each field was measured.

** For the salmon, the height of the epithelial cells was taken in the center of each field where vacuole and nuclear counts were made. For each trout, fifty epithelial cells were chosen at random.

*** Comparative diameters of the duct lumens made by taking the average of the widest horizontal and vertical diameters.
iron hematoxylin. The vacuoles are either apical or lateral to the nucleus. Many of the nuclei have lost their circular outline and have become sickle-shaped. Sections of the urogenital sinus show no signs of vacuolization in the squamous epithelial cells.

Extra-testicular ducts from the matured March and April trout are also distended with milt. The epithelium varies from columnar to cuboidal in a single section. There was no significant difference between the epithelial cell heights measured from trout taken in March and trout taken in April. The nuclei of the epithelial cells are nearly all centrally located. There is no sign of vacuolization nor of secretion in the epithelium.

In the post-spawning salmon, the extra-testicular duct contains a few sperm (Fig. 16). The average epithelial height is approximately the same as in the spawning fish. The size of the vacuoles average 1.3× larger in diameter than in the spawning salmon. The vacuoles are fewer in number than in the spawning salmon, but a number of the vacuoles seen to have fused and many of them have broken. In places along the duct wall the epithelial cells have undergone cytolysis so the vacuoles are obliterated. Most of the epithelial nuclei are shrunk in various stages of pyknosis. The cytoplasm takes a more basic stain than in the spawning salmon. The duct connective tissue of the salmon with the more badly rotted fins and the gut also shows signs of necrosis.
For the post-spawning trout, the extra-testicular duct lumen contains a number of sperm which do not show signs of disintegration (Fig. 17). The epithelial cells have the same appearance as those in the spawning trout. The height of the epithelium is approximately the same as in the spawning trout. The nuclei are normal and centrally located in the cell. There is no indication of vacuolization nor of necrosis in the extra-testicular duct epithelium of the post-spawning trout.

(b) Discussion: The male sockeye salmon and rainbow trout have the type of genital ducts which appear to have no connection with the kidney ducts. The genital ducts of the trout and salmon are similar excepting for the changes which occur in the epithelial cells of the salmon extra-testicular duct on the approach of sexual maturity. These changes are similar to the changes occurring in the testes of the salmon.

The epithelial cells from October salmon resemble secretory cells in some respects. The cells are tall column and most of their nuclei are basal. The vacuoles appear as though they may be secretory vacuoles. However, in spawning salmon, when the vacuoles are most numerous, there is a reduction in the height of the epithelial cells to less than half that of the October salmon. Also the vacuoles increase markedly in size and no longer resemble secretory vacuoles. Yet this is the stage in which one
would expect to find the greatest amount of secretion. In
the spawning salmon the distention of the duct lumen with
sperm at least partially accounts for the reduction in the
epithelial cell heights.

The post-spawning salmon have fewer vacuoles than
spawning salmon, since many of the vacuoles have fused,
others have broken, and cytolysis obliterates some. The
average epithelial cell height remains about the same as
in the spawning fish, and the duct lumen show only a
slight contraction, even though the greater mass of the
sperm have been expelled. The vacuoles are even larger
than they are in the ducts of the spawning salmon and are
definitely a part of the degeneration which is taking
place in the epithelial cells.

The epithelial cells of the trout's extra-testicular
duct exhibit no necrotic stages and vacuoles are lacking
in all the stages studied. During their spawning and
about a month after the trout have been stripped, the
epithelial cells of the ducts show no definite signs of
secretion.

The vacuoles present in the salmon’s duct epithelium
are interpreted as a sign of necrosis rather than of
secretion.

Summary

1. The testis development in the rainbow trout and
sockeye salmon is of the radial type. Nests of spermatogonia
are separated by strands of connective tissue. Secondary ducts are branched off from a main dorsal testicular duct, and the lumina of the secondary ducts connect with the lumina formed in the nests of germ cells. The germ cells are thus incorporated into a complexly branching tubular system.

2. The adult salmon testes are essentially similar to those of the adult trout. The testes are divided by connective tissue partitions into complicated lobules which empty into a main dorso-radial testicular duct. The point of the mesorchium's attachment to the testis is changed by a rotation of the testis during its increase in size from its dorsal position, as found in the mature testis, to a dorso-radial position.

3. During maturation the lobules of the salmon testes are packed with cysts of germ cells in various stages of spermatogenesis. The cysts break in the spermatid or spermatogonia stages thus giving the lobules a patent lumen containing a loose mass of germ cells. Vacuoles occur in many of the epithelial cells of the extra-testicular duct in the salmon at this stage.

4. In the spawning season the testes and the genital ducts of the salmon and trout are distended with milt. The salmon testes at this time have vacuolization and pycnosis occurring in the epithelial cells lining the ducts, in the spermatogonia, and in the follicle cells. This condition is completely absent in trout testes of approximately the same sexual development.
5. After the spawning season, the epithelial cells of the testes' ducts, the spermatogonia, and the follicle cells in the salmon are definitely necrotic, having pycnotic nuclei and large vacuoles. Neither vacuolization nor necrosis occur in cells of the post-spawning trout testes and genital ducts. The vacuolization in the cells of the salmon testes are due to necrosis, and the necrotic changes are a sign of the approaching death of the organism.

6. Sclerotic spermatogonia, bare no spermatocytes or spermatids, are present in the spawning and post-spawning trout. There are enough spermatogonia remaining after the spawning period to replenish the testes with sperm for successive spawning seasons. The salmon resemble the trout in this respect, excepting that the sclerotic spermatogonia continue degenerating during the spawning season.²

²Present in sections of ovaries from spawning salmon are a few undeveloped oocytes and a number of scattered cells, which resemble the primary spermatogonia.
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Fig. 2. Immature hatchery raised male sockeye salmon, three years of age. Length, twelve centimeters.

Fig. 3. Mature male sockeye salmon at time of spawning, four years of age. Length, twenty-eight centimeters.

Fig. 4. Cross-section of an immature salmon testis. X 75. a.b.l., portion of air bladder; b.l.v., blood vessel; c., capillary; int.m.s., intermesorchial space; n.g., nest of germ cells; sec.t.d., secondary testicular duct; t.d., testicular duct.

Fig. 5. Portion of the same section. X 275. c., capillary; m., germ cell in active mitosis; n.g., nest of germ cells surrounded by follicle cells.
PL. IX. II
Explanation of figures

Fig. 6. Cross-section of testicular duct in immature salmon testis. × 375. bl.v., blood vessel; g.c., germ cells lining secondary testicular duct; l., lumen in germ cell cast; sec.t.d., secondary testicular duct; t.d., testicular duct.

Fig. 7. Cysts of germ cells in various stages of maturation. October salmon testis. × 550. sc., cyst of spermatocytes in synizesis; sp., spermatzoa; st., cyst of spermatids.

Fig. 8. Section of testis from spawning salmon showing non-vacuolated spermatogonia. × 550. See also Fig. 12. con.t., connective tissue; r.s.j., resting spermatogonia; sp., spermatzoa.

Fig. 9. Section of testis from spawning trout which contains an unusually large number of resting spermatogonia. × 550. Note prominent nucleoli. con.t., connective tissue; r.s.j., resting spermatogonia; sp., spermatzoa.
PLATE III
Explanation of Figures

Fig. 10. Section of testis from spawning salmon.
November. X 400. cont., connective tissue of lobule septa; sp., spermatozoa; v.s., vacuolated spermatogonia.

Fig. 11. Section of testis from post-spawning salmon. January. X 400. py.n., pyoctic nucleus of spermatogonia; sp., spermatozoa; v.s., vacuolated spermatogonia.

Fig. 12. Section of testis from post-spawning salmon. January. X 400. No good sections can be made of this tissue due to its acerotic condition. 114., lipid from cytolsysis of epithelial and germ cells; s, cont., connective tissue of lobule septa.

Fig. 13. Section of testis from post-spawning trout. May. X 400. cont., connective tissue of lobule septa; v.s., restin spermatogonia; sp., spermatzoa.
PL. T. IV

Explanation of Figures

Fig. 14. Section of extra-testicular duct from an October salmon. X 75. The numerous spaces basal to the epithelium are filled with lymph.

Fig. 15. Section of extra-testicular duct from a spawning salmon, November. X 75. The duct is distended with spermatozoa. Notice the numerous vacuoles in the epithelial cells.

Fig. 16. Section of extra-testicular duct from post-spawning salmon, January. X 75. Notice the large vacuoles occurring throughout the epithelium.

Fig. 17. Section of extra-testicular duct from a post-spawning trout, May. X 75. No matter of sperm remain in the lumen. There is a complete absence of vacuoles in the epithelial cells. A large lymph space is present in the right-central portion of the section.