T H E S I S

On

SEASONAL CHANGES

in the

GONADS OF THE PACIFIC COAST NEWT

NOTOPHTHALMUS TOROSUS ESCHSCHOLTZ

Submitted to the

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INTRODUCTION

The Pacific coast newt or "water-dog" as it is commonly called has a very wide distribution. It is found all the way along the Pacific coast drainage from southern Alaska to southern California and is chiefly confined to the upper Sonoran and transition life zones (Grinnel and Camp '17). The optimum conditions for its existence are regions which have moderate variations in climate the year round. Such are the conditions about Corvallis where the newt is found in abundance.

The purposes of the present investigation have been three-fold. In the first place, it has been carried out with the idea of determining the seasonal variations which occur within the gonads. In the second place, the object has been to ascertain the correlation which exists between the germinal and somatic changes, and lastly, to note the seasonal variations in the behavior of the newt.

Seasonal changes in the gonads of various forms have been described by numerous investigators of gametogenesis, but few have taken up the work with the idea of seasonal variations in mind. Previous investigation seems to have been confined to the study of germ cells with the idea of finding good cytological material for the purpose of elucidating the various steps in the maturation of the germ cells. There is a wealth of literature on the

cytology of amphibians particularly from the group of urodeles, to which the newt under consideration belongs.

Previous work on Notophthalmus torosus has involved the mentioning of seasonal changes. Cope ('89) has given a description of the external appearances of the newt. Ritter ('98) has dealt chiefly with its life-history and habits. Lebrun ('02) has described the maturation process for the germ cells of the female. Chandler ('18) has taken up the relation of the western newt to mosquito control.

The writer is greatly indebted to Dr. Nathan Fasten, head of the Department of Zoology and Physiology, and Dr. Florence Hague for their kindly interest and criticism.

MATERIAL AND METHODS.

During the course of my investigation I have confined myself chiefly to the sexually mature adults. These are found in temporary and permanent ponds and slowly flowing streams for the greater part of the year. During the late fall and winter months, the animals hibernate on land often at a considerable distance from the water. Here they may be found under old logs, at the base of trees, and under debris.

The material for this work has been collected from three localities about Corvallis: (1) Colorado Lake,

(2) Mary's River Dam, and (3) one mile west on Oak Creek. Specimens have been collected for a period of one and one-half years at intervals from one to two weeks apart. The gonads were fixed either in the field or immediately upon arrival in the laboratory. Owing to the fact that the author was absent from Corvallis during the summer months, material was not available for this season of the year, from June to August. Inasmuch as Lebrun ('02) has described the oogenesis and changes in the female germ cells, I have confined my observations chiefly to the internal changes in the gonads of the male.

Various fixatives were employed for the preservation of the testes. The best results were obtained with the following fluids: Bouin's, Ezra Allen's modification of Bouin's, and Flemming's mixture. Sections of the testes and also of various somatic tissues were cut from five to twelve microns thick, and stained with Heidenhain's iron hematoxylin, safranin and gentian violet, or Delafield's hematoxylin and eosin. Smear preparations of the testes, fixed and stained in the above manner, were also available.

DIFFERENCES BETWEEN MALE AND FEMALE.

The male newt leads more of an aquatic life than the female. In many instances the terrestial life of the male is lacking, but it may be said that the period of

hibernation is approximately from the middle of October
to the first of January. As early as the latter part of
December and until the following fall the male is aquatic.
On the other hand, the female is found on land from
October to the middle of February and then she migrates
into water to deposit her eggs. She probably remains
here until the coming fall. The intense mating season
commences as soon as the females take to the water and is
at its height during the months of February and March.

The breeding season is the period of the year when
the male and female newts show their external differences
to a marked degree. The female has a narrow tail and her
skin is rough and papillated, except along the abdomen,
where it is smooth and thin owing to the fact that this
region is distended with maturing eggs. The male has a
smooth, soft skin; a widened tail, enlarged cloacal lips,
and a dark band of pigmentation extending from the region
of the pectoral girdle to the cloacal lips. There are
also horny pigmented areas on the inner surface of the
legs as well as papillated skin on the ventral surfaces
of the abdomen and mandible. The tips of the digits may
be blackened. This, however, is a less constant feature.

Following the breeding period and during terres-
tial life the two sexes resemble each other very closely.
This similarity is so marked that Ritter ('89) thinks it
impossible to distinguish the male from the female. At

this time both individuals possess the roughened, papillated skin and narrow tails. There are however, certain distinct differences which reveal themselves upon close examination. The body length of the male averages eighteen centimeters while that of the female is thirteen centimeters. The body weight·of the male is approximately one-third greater than that of the female. The skeletal framework of the male is constructed more substantially. The most important means of distinguishing them is by aid of differences in the appearance of their cloacas. The female cloaca is less prominent, it appears as a ridge upon which is located an aperture that measures from one-half to three-quarters of a centimeter in length. The male cloaca is prominent and rounded laterally, centrally is the aperture which is from one to one and one-half centimeters in length.

The morphology of the male cloaca for the European Triton has been described by Heidenhain ('90). The cloaca of Notophthalmus torosus closely corresponds to that of its European relative. In Notophthalmus torosus the male cloaca (text fig. A) consists of two portions, (1) a cloacal chamber (fig. A, cc) which is ectad (towards exterior) and caudo-ventral, and (2) a cloacal tube (fig. A, ct) which is entad (towards interior), more dorsal and cephalic. There are three glands within the region of the cloaca, (a) a large cloacal gland

Text Figure A.

Diagrammatic sketch of a longitudinal
section through the male cloaca. Ant., anterior;
Post., posterior; ag, abdominal gland; cc, cloacal
chamber; cg, cloacal gland; ct, cloacal tube; pg,
pelvic gland; te, tongue-like elevation. X 8.5.

Text Figure B.

Transverse section through the female
cloaca. cc, cloacal cavity; me, mesal elevation;
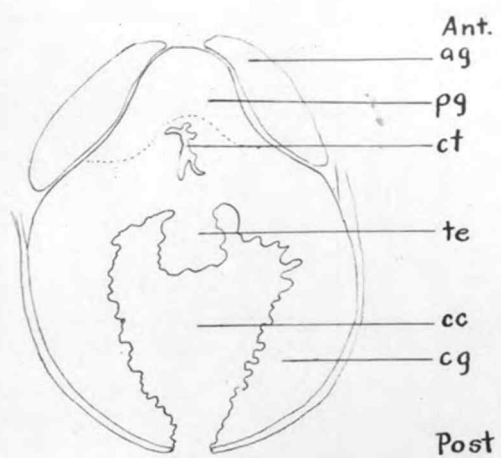sp, spermatheca; vg, ventral gland. X 40.

Ant.
ag
pg
ct

te

cc
cg

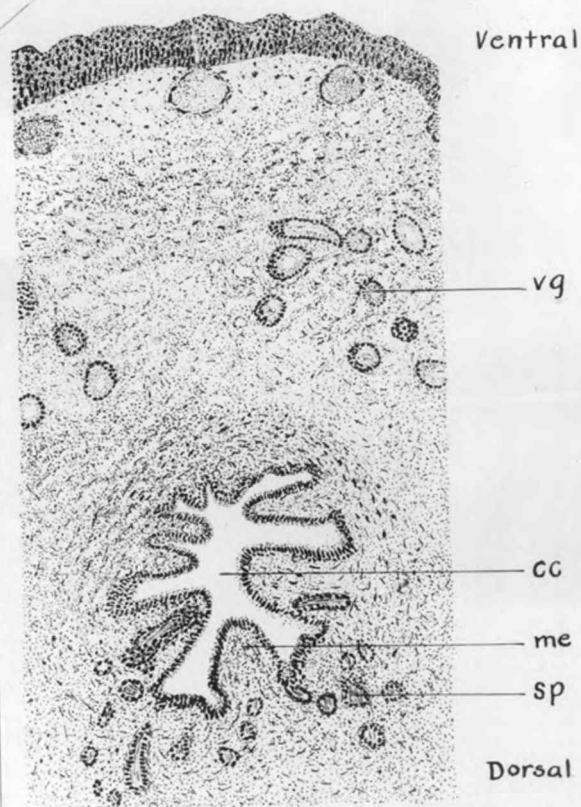Post

Figure A. x 8.5

Ventral

vg

cc

me

sp

Dorsal

Figure B x 40

(fig. A. cg) which forms the basis of the cloacal walls
and opens into the cloacal chamber upon the summits of
longitudinal folds, (b) a smaller pelvic gland (fig. A,
pg) on the dorsal side of the cloaca, and (c) an abdo-
minal gland (fig. A, ag) located in the abdomen between
the abdominal muscles and peritoneum. Situated in the
cephalic portion of the cloacal chamber is a "tongue-like
elevation" (fig. A, te), which is attached dorsally and
presents a free knob-like end ventrally. This was incor-
rectly termed "penis" by Heidenhain ('90) and was given
the name of "cloacal papilla" by Blanchard ('81). Kings-
bury ('95) thinks the latter term confusing and calls it
a "tongue-like elevation." This is a persistent cloacal
structure with some degree of contraction and increase
in vascularity during amplexus. Although no function has
been attributed to it, it may be indirectly of some use
in the expulsion of spermatozoa or during the act of
amplexus.

The morphology of the female cloaca (text fig. B)
corresponds to that described by Kingsbury ('95) for the
eastern newt Notophthalmus viridescens. In this form, he
finds only one cloacal cavity (fig. B, cc) with simple
lips and no longitudinal folds. There is a mesal eleva-
tion (fig. B, me) dorsally which divides that portion of
the cavity into two parts. The glands of the female
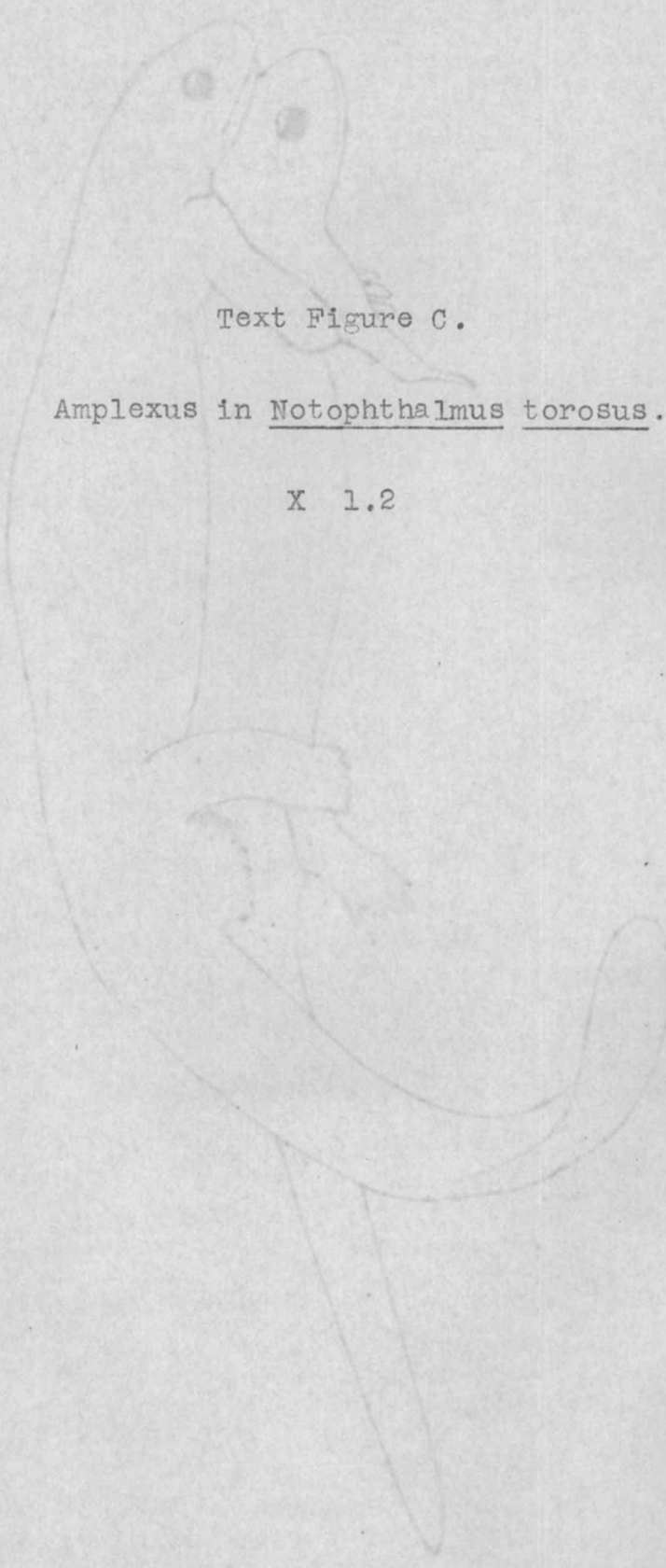cloaca are: (a) a ventral gland (fig. B, vg) which opens

into the skin at the edge of the aperture, and (b) some
forty to fifty flask-shaped tubules situated dorso-
laterally which open into the cavity on either side of
the mesal elevation. These latter structures are the
spermathecae (fig. B, sp) which retain the spermatozoa
within the cloaca and thereby insure internal fertiliza-
tion.

BREEDING HABITS

a.Amplexus

Amplexus takes place sparsely in the fall and
winter months, but with the approach of spring, during
February and March, pairs of copulating newts are numer-
ous. From the evidence one would conclude as Ritter
('89) suggests that the autumnal mating season is to be
regarded as a preparation for the more intense season
which soon follows.

During the act of amplexus the male clasps the
female with his hind and fore limbs so that the fore feet
meet ventrally and the hind legs may be in contact with
the cloacal lips of the female (text fig. C). The en-
larged cloaca of the male straddles the tail of the
female. In this position the animals either swim about
or rest on the bottom of the pond. The widened tail of
the male propels the pair through the water by powerful
strokes. The duration of a single act of amplexus varies

Text Figure C.
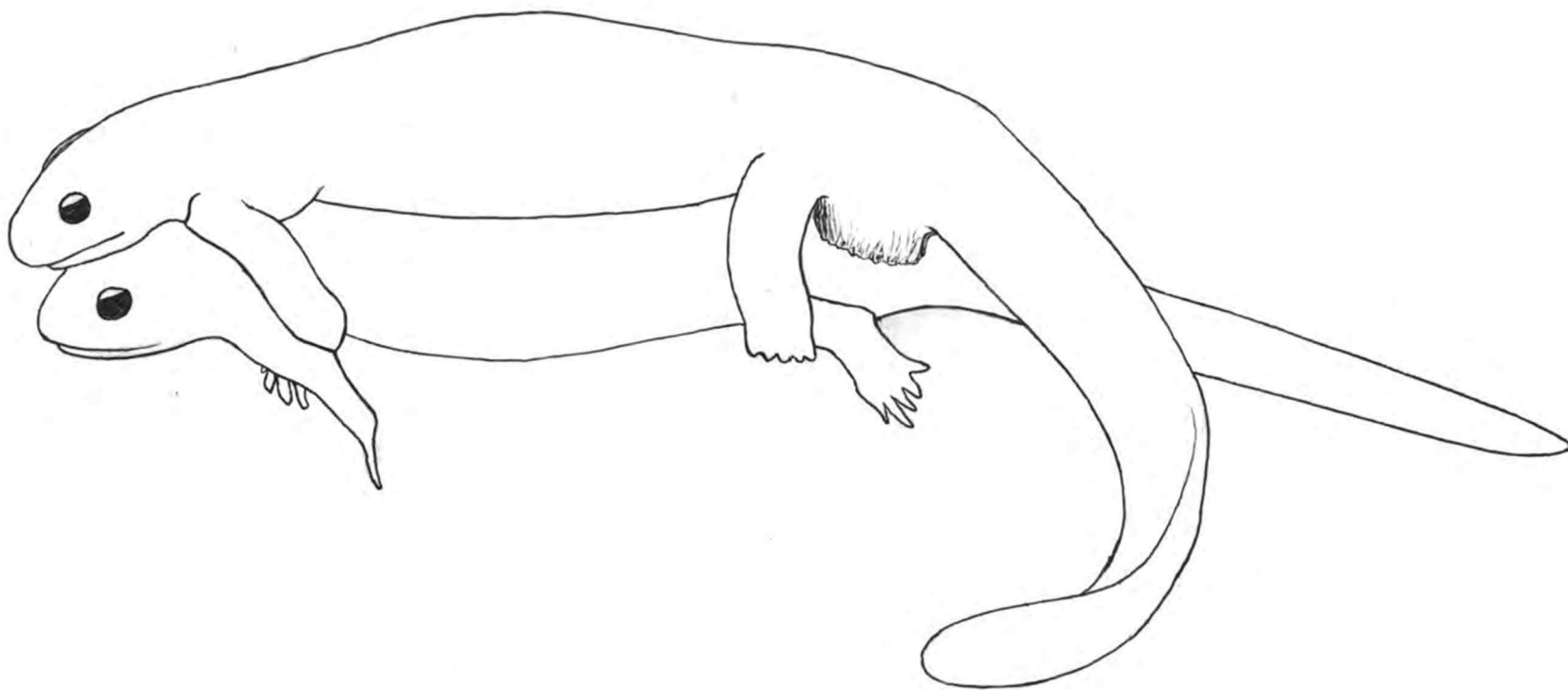
Amplexus in Notophthalmus torosus.

X 1.2

Figure C   x1.2

from one to twenty-four hours. Occasionally the male
has been noticed to grasp the female in such a way that
there is direct cloacal contact. However, this is not
the normal position.

From observations in the laboratory it has been
impossible to determine the exact means by which the
spermatozoa of the male reach the cloaca of the female.
The male newt of the eastern United States Notophthalmus
viridescens deposits stalked masses (spermatophores)
which contain spermatozoa. The deposition of these
structures takes place following amplexus. They are
later taken up by the cloaca of the female. It is known
(Ritter '98, Chandler '18) that no spermatophores are de-
posited by the male of Notophthalmus torosus. In a few
instances masses of spermatozoa have been taken from the
cloacal cavity of a female which has been in copulation.
The evidence seems to point to the fact that undoubtedly
impregnation of the female of Notophthalmus torosus takes
place during amplexus.

There are certain interesting manoeuvers on the
part of the male when copulating, such as a rubbing of
the ventral border of the mandible against the dorsal
surface of the head of the female or an occasional twitch-
ing of the hind legs as the pair come to a resting
position. The last mentioned act may have something to
do with the transference of the mass of spermatozoa.

b. Egg-laying.

The period of egg-laying follows the mating
season or is continuous with the latter part of it.
Eggs were first deposited in the vicinity of Corvallis
about the middle of March. The newt lays her eggs
singly or in groups of two or three on water plants,
sticks, or debris at the bottom of the pond. This con-
firms Chandler's ('18) observations on the number of
eggs deposited and is in accordance with what happens in
the eastern species Notophthalmus viridescens as des-
cribed by Jordan ('93). However it is in contradiction
to what has been observed for the same species of newt
in California, namely, that clusters of eggs are deposi-
ted.

The places selected for the deposition of eggs
are shallow ponds or slow-flowing streams overhung by
willows and low bushes. The female clasps the stem of
the plant between her hind feet, depresses the posterior
portion of the abdomen so that the cloaca is in contact
with the plant and then gradually raises this region as
the egg is deposited. This position is generally as-
sumed for ten to fifteen minutes during which time the
animal clings tenaciously and is not easily frightened.
In nature the egg-laying season in a single female pro-
bably lasts over a period of from four to five weeks as
Jordan ('93) has suggested in the case of Notophthalmus

viridescens.   When the animals are transferred to the
laboratory they cease to lay eggs after ten days.

c. Reflexes.

A male which has developed the secondary sexual
characteristics of the breeding season will clasp a stick
if it is placed between the hind legs. Such behavior
corresponds to the clasping reflex of the fore-legs of the
frog (Goltz '65). It was further noticed that when skin
between the hind legs was rubbed with the finger the male
would clasp it. If the skin were removed in this region
the animal would not respond to a stimulation of the un-
derlying tissue. Furthermore if the spinal cord were
sectioned midway the clasping reflex still persisted.
From these results it is safe to conclude that (1) the
nervous center for this reflex is located fairly low with-
in the spinal cord, and (2) the skin contains the neces-
sary sensory receptors for bringing about the reflex. A
female at the time of egg-laying can occasionally be made
to clasp one's finger with her hind feet. All endeavor
on my part to determine the nature of such behavior has
proven unsatisfactory. This reaction seems less constant
than the clasping reflex of the male and in many instances
is not noticeable.

A finger rubbed across the ventral surface of the
abdomen of a decapitated male causes the animal to arch

its abdomen dorsally. Here, as in the clasping reflex,
the removal of the skin destroys the reflex.

As already pointed out, during the act of amplexus
the male shows certain interesting movements. The ven-
tral border of the mandible is rubbed against the dorsal
surface of the female's head, with the result that the
floor of the mouth is depressed and occasionally the mouth
is opened. Destruction of the skin in this area results
in a disappearance of the behavior. There seems to be a
seasonal variation for the intensity of all of these re-
flexes, for they become less decided following the mating
period and in some instances seem to entirely disappear.

SEASONAL CHANGES
a. Behavior

Thigmotaxis. Both sexes are positively thigmotactic at
all seasons of the year. This is, however, more decided
in the female than in the male. The period of hiberna-
tion, when the animals crawl under old logs, debris, etc.,
is a manifestation of this response. It is probable that
temperature plays an important role in the thigmotactic
responses. Torelle ('03) has shown in the case of the
frog that when the temperature is lowered the reaction
becomes decidedly positive. Such is the case in Notoph-
thalmus torosus; during the warmer days of the hiberna-
tion period they are found actively out in search for
food, whereas on colder days, they are inactive and hid-

den. The female during the egg-laying period is found among the tangled water-weeds laying her eggs. At the same time few, if any, of the males are to be found in this region of the pond.

Phototaxis. The animals orient themselves so that the head end is towards the source of light and are thus positively phototropic. Temperature may also effect this response. The evidence seems to point to the fact that as spring approaches and the days become warmer and lighter the animals become more decidedly phototropic. The colder days during hibernation finds them negatively phototropic because on the bright cold winter days few of them are to be found out from their hiding places.

b. Somatic changes.

The external appearances of the secondary sexual characteristics of the male have already been enumerated. There remains to be discussed a histological description of the smooth skin of the male of the breeding season as compared to the roughened, tuberculated skin of the individual of the hibernation period. There are two apparent differences, first, the smooth and thickened epithelial layer is in contrast to the uneven and thinner layer of the rough male, and secondly, the marked increase in size of the layer of connective tissue underlying the glands is

correlated with the thickened condition of the epithelium.
Freshly cut skin of the male in the smooth condition shows
the connective tissue layer to have a milky, semi-trans-
parent appearance and a gelatinous consistency. Chemically
it is rich in mucin as shown by the positive glyco-protein
tests for this substance. The possibilities of the origin
of the mucin are several: (1) from the connective tissue
cells making up the layer, (2) from the mucous glands of
the skin, and (3) from the cloacal glands. A more exten-
sive study of the nature of this condition would probably
reveal the origin of the mucin.

There are additional changes in the epithelium
which correspond closely to those described by Kingsbury
('95) and Gage ('91) for Notophthalmus viridescens. The
lateral sense organs of Notophthalmus viridescens have
during the terrestial life, a thin covering of epithelium
which disappears as the aquatic life is assumed (Kings-
bury '95). Sections through the skin from the regions of
the lateral sense organs show such changes in Notophthal-
mus torosus. The layer of epithelium is undoubtedly for
protection of these organs while the animal is on land.
The change in the western newt is seasonal but in the
eastern representative it is permanent, because once
Notophthalmus viridescens assumes an aquatic life it
does not return to the land. Gage ('91) first mentioned
the change from ciliated to non-ciliated epithelium in

the oral cavity of Notophthalmus viridescens as this newt
passes from a terrestial to an aquatic life. On this
point my observations confirm those of Ritter ('97) who
reports a similar condition for Notophthalmus torosus,
but with many striking exceptions.

c. Changes in the male gonads

1. Morphology of the male gonads.

The testes are a pair of light yellow, sub-divided
bodies (fig. 1, t) suspended from the dorsal body wall by
folds of peritoneum. Vasa efferentia connect each testis
with the median border and anterior portion of the meso-
nephros (fig. 1, mm'). Ductules pass out from the kidney
to the Wolffian duct (fig 1, w) which in turn opens into
the cloaca (fig. 1, o). Spermatozoa are produced in the
testis and pass out into the vasa efferentia, through the
mesonephros, into the coiled Wolffian duct which serves as
a conveyor of both urine and spermatozoa.

Each testis is divided into triangular lobes which
are connected by a cord of germinal epithelium (fig. 1,
g). The largest of the divisions is located anteriorly
while the smallest is posteriorly. The number of the lobes
is generally constant for opposite sides of the coelom. If
there is a variation it is seldom more than a difference
of one. The males with the greater body weight seem to
have more lobes to the testis than those which are light-

er. The reasons for these variations may be various, such as differences in seasons of sexual maturity, or of food, temperature and the like. Each lobe of the testis is enclosed within a connective tissue capsule and this is depressed ventrally. Septa pass into the testis from this point and radiate through the testicular substance dividing it into lobules. Each lobule is further divided during the process of meiosis into cysts.

There is within each lobe of the testis a distinct progression of the stages in spermatogenesis (fig. 2). Anteriorly (fig. 2, A) are the dividing spermatogonia (fig. 2, sg), while posteriorly (fig. 2, P) are the spermatozoa (fig. 2, sz) and in between these two regions are the intermediate stages (fig. 2, ps, ss, sp). Such an arrangement is of value in interpreting certain of the steps in maturation, especially the heterotypic prophases.

2. Seasonal variations in the testes.

The testes show a marked variation in dimensions for different parts of the year. Turner ('19) states that the variation in volume of the amphibian testes is less pronounced than for the teolost. Due to the lack of equipment I have been unable to determine the variation in weight during the year, but from a measurement of lengths and widths the following conclusions have been reached: (a) The maximum length of the testes is attained

in late December or early January. At this time the tes-
tes are probably the heaviest. (b) Toward the middle of
the mating season, about the first part of March, the
length decreases materially and the weight of the gonads
is probably the least. (c) The width of the testes is a
less variable factor than the length. The average length
of the anterior lobes of the testes for twenty-five speci-
mens, during the period from September to the first part
of January, was 6.8 mm., and the width 4 mm. From the
latter part of February and up to the present writing
the average length has been 4 mm., and the width 4.5 mm.

There are numerous internal changes in the testes
which are correlated with the above volumetric variations.
During the late fall and winter months the posterior
half of the testes becomes filled with immature sperma-
tozoa. Immediately following the complete expulsion of
the spermatozoa, the testes attain their minimum size.
Going hand in hand with these changes there occurs a pro-
liferation of the immature germ cells. Because of this
the volumetric variation in size is not so marked as if
there were a separate period between the release of the
spermatozoa and the proliferation of spermatogonial cells.
Not having material for the summer months it is possible
only to predict the course of events within the testes.
One would expect to find abundant mitoses and transform-
ing spermatids at this time.

3. Observations on spermatogenesis

(a) Spermatogonial stages

The location of the spermatogonial stages is re-
stricted to the anterior end of each lobe of the testis
which is periodically supplied from the cord of germinal
epithelium. The spermatogonia may be readily distin-
guished from the other stages within the testis because
of their lightly staining capacity, their characteristic
nuclei, and the large follicle cells which surround the
outside of their cell walls. The cytoplasm occasionally
appears to be obliterated by the huge nucleus, which
presents an irregular shape. The portion of the nucleus
toward the centrosome is lobulated while distally the
lobes are fused (fig. 7). Such an appearance is spoken
of as polymorphism and Wenrich ('16) has shown in case
of the grasshopper, Phrynotettix, that each of the lobu-
lations represents a chromosome which has become alveoliz-
ed and has formed a vesicle about itself.

The internal structure of the nucleus presents an
irregular linin network with considerable karyolymph,
and chromatin granules with two or more nucleoli.

Prior to 1900 the general opinion of investigators
on amphibian cytology was that amitotic division took
place within the spermatogonia (St. George '85, Meves
'91, Benda '93, and McGregor '99). Later opinions on the

nature of these divisions confirm the idea that it is mi-
totic. My own observations on the spermatogonia of
Notophthalmus torosus lead me to conclude that the divi-
sion of the spermatogonia is mitotic, although it is
scattered and takes place rather rapidly. As successive
stages are produced changes in the appearances of the
cells can be noticed (figs. 3-6). They decrease in size,
the nucleus becomes smaller and stains a deeper hue, and
most of the chromosomal vesicles disappear. The follicle
cells become flattened; they no longer surround one
spermatogonium but several. If the size of the spermato-
gonia and the changes which occur within the nucleus are
the factors by which the number of generations are to be
determined then there are approximately four divisions in
the species under consideration.

Twenty-four chromosomes may be regarded as the
diploid number for Notophthalmus torosus which can be
counted in polar views of either the somatic (fig. 8)
or spermatogonial metaphases. This count verifies the
number mentioned by Lebrun ('02) for the female of the
species, although Parmenter ('20) working with Ambystoma
tigrinum considers Lebrun's count only approximate.
Twenty-four chromosomes is generally regarded as the
number present in the somatic cells of Salamandra and
Triton. The diploid number of twenty-eight has been re-
ported for some of the other urodeles as Aneides lugubris

(Snook and Long '14) and Ambystoma tigrinum (Parmenter '20).

b. Primary spermatocyte stages

Following the telophase of the last spermatogonial division is the resting stage of the primary spermatocyte which is comparatively short in duration. The nucleus at this time is spherical and the chromatin is in the form of clumps or short blocks (fig. 9). The subsequent stages of the primary spermatocyte are important because there is a reduction and redistribution in the number of chromosomes as well as of the hereditary units. The various steps will be traced logically.

Prophases (figs. 9-15.) The nucleus undergoes growth and the blocks of chromatin become connected by linin threads. It is possible now to count these clumps (fig. 9). There are twenty-four of them, being the same number found in the diploid number of chromosomes. Wenrich ('16) thinks that each block gives rise to one chromatin thread. In Notophthalmus torosus the stage following the disappearance of the chromatin lumps and the appearance of tangled threads is confusing, evidently resembling the condition described by Swingle ('21) for the germ cells of the larva of Rana catesbiana. The threads however are seen to loop themselves so that the free ends are toward the centrosome (fig. 10). They have a mossy or woolly appearance due to the distribution of the chromatin

granules.

Following the leptotene period just described
there is a transitional stage which has been termed the
amphitene by Janssens ('95). The threads come together
side by side (parasynapsis) and fusion begins in the re-
gion of the nucleus nearest the centrosome and gradually
proceeds distally (fig. 11). Simultaneous with this
fusion there also occurs a twisting of the threads. All
evidence of the double origin of the resulting unit is
lost. These observations confirm those of Janssens ('05)
and Wilson ('12) who report for Batrocoseps a fusion of
two threads only, to form the pachytene thread.

Synizesis has been described as a normal condition
in the prophase of the primary spermatocyte of Bufo by
King ('07). Janssens ('01) considered it a definite stage
in the Triton but later ('05) he recalled his previous
opinion. A study of my preparations does not reveal a
stage in which the nuclear contents are contracted to one
side of the nucleus and I am of the opinion that it does
not occur in the western newt under discussion. It is
without doubt an important phenomenon in the history of
germ cells of other forms.

The pachytene bouquet consists of loops of the
pachytene threads with their free ends oriented toward
the centrosome (fig. 12). In the case of Batracoseps
(Janssens '05), the nuclear contents turn so that the

loops are at right angles to the centrosome. This has
been observed in Notophthalmus torosus but whether or
not it is a constant feature the writer cannot say. Soon
the threads lose their woolly appearance and become more
condensed so that the granules of the chromatin are less
pronounced than in the preceding stages.

Reports on the splitting of the pachytene threads
are confusing. Montgomery ('11) states that the double-
ness of the threads may be seen at all times. The
Schreiners ('05, '06) are of the opinion that it disappears
for a time but later reappears. In this form the fusion
of the leptotene threads may not be complete but there is
no line of demarkation between them after parasynapsis
has taken place. As the primary longitudinal split occurs
the threads do not separate at their extreme distal ends
but remain together (Janssens '05), the split gradually
proceeding proximally. This prediplotene condition (fig.
13) may be very easily confused with the stage of parasyn-
apsis in which the distal portions of the threads come
together later than the proximal ends. Due to the seria-
tion of stages within the testis of Notophthalmus torosus
(fig. 2) the pre- and post-synaptic stages may be readily
distinguished.

The prediplotene as well as the following period
marks an increase in the size of the nucleus. The threads
become shorter and consequently thicker. They twist about

each other, although the twisting is not as thorough as
during synapsis (fig. 14). A second split follows along
the length of the threads, thus being longitudinal, simi-
lar to the first cleft. As a result, there is formed
the tetrad-complex of four chromatids with their ends to-
gether. The threads then open out along the primary and
secondary splits in a manner so as to form such figures
as 8's, X's, and V's. Condensation of these structures
begins and apparently is soon completed. This opening
process continues with the result that there is formed
the ring-shaped tetrads (fig. 15) described by investi-
gators of amphibian spermatogenesis. Simultaneously with
the formation of the tetrads there is the further in-
crease in the size of the nucleus.

Metaphase (fig. 16). The centrosome divides at some in-
terval during the late prophase and the halves pass to
opposite poles of the nucleus. The nuclear membrane dis-
integrates and the tetrads become arranged on the equator
of the spindle. Twelve chromosomes may be counted in a
polar view of such a stage.

Anaphase (fig. 17). The two univalent components of the
tetrads separate in the form of V's and J's and move to
opposite poles. This is followed by a fusion of the
chromosomes into a solid mass.

Telophase (fig. 18). A line of constriction appears
midway between the two dark masses and gradually divides

the cell. A nuclear membrane is formed about the dis-
integrating chromatin masses. As a result of these
intricate steps which the primary spermatocyte undergoes,
two types of secondary spermatocytes arise which will be
explained presently.

(c) Secondary spermatocyte stages.

The mitotic division of the secondard spermatocyte
is equational. Here the resting stage is extremely
short. In some instances, immediately after the forma-
tion of the nuclear membrane the contents of the nucleus
disintegrate and the chromosomes of the secondary sperma-
tocyte are formed.

Counts of chromosomes as seen in polar views of
metaphase stages show that there are two types of secon-
dary spermatocytes produced, those with eleven chromo-
somes (figs. 19 and 20) and those with twelve (fig.21).

There are no tetrads formed as in the previous
division, the chromosomes are smaller in dimensions (fig.
22). They separate along the longitudinal split and pass
to opposite poles in the form of V's, one arm of which
may be longer than the other. Condensation of the chro-
mosomes soon occurs and formation of a nuclear membrane
with constriction of the cell to form two spermatids.

(d) The spermatid stage

The spermatids (fig. 23) are present within the lobes of the testis for a considerable length of time. Inasmuch as suitable material for the study of the transformation of the spermatid into the spermatozoon has not been available, this phase of the problem has not been worked out.

DIMORPHISM OF THE SPERMATOZOA

The present literature on the dimorphism of the spermatozoa of amphibia is exceedingly scarce. King ('12) reports dimorphism in the spermatozoa of Necturus maculosus. Zeleny and Faust ('15) report a dimorphism in the spermatozoa of Rana pipiens, based largely on a comparison of the length of the head-pieces of the spermatozoa. Swingle ('17) described an odd chromosomal body in the germ cells of Rana pipiens as an accessory chromosome. He has later ('21) been in doubt as regards the nature of this structure but thinks it might probably be a "precociously dividing chromosome," one-half of which migrates more readily to one pole than does the other half. Swingle states that Levy ('14 and '15) in an unpublished article reports a chromosome in Rana Temporaria similar to the one described by himself.

There is within the germ cells of Notophthalmus torosus in the heterotypic metaphase a chromosome which

is found to approach one pole of the nucleus while the remaining chromosomes are still in the equatorial plate (fig. 16). This body is nearly identical with the one described by Swingle ('17) and undoubtedly this latter investigator would probably interpret it as a "precociously dividing chromosome." Having been unable to trace its history I cannot definitely interpret it otherwise.

King ('12) describes a multiple chromosome in the heterotypic maturation mitosis of Necturus with terminal projections at right angles to the main portion. One of the projections is longer than the other and often appears attached to the bivalent chromosome by fine threads. She regards such a structure as the "x-chromosome." In Notophthalmus torosus there is seen in the early anaphase of the first maturation division a figure which has the appearance of two J-shaped chromosomes dividing (fig. 17). The terminal portions of these elements are bent at right angles to the main axis, but there is no difference in size to be recognized between them.

In the secondary maturation mitosis there is a chromosome (fig. 22) which behaves similarily to the "x-chromosome" found in Necturus maculosus as shown by King ('12). It appears on the spindle during the early anaphase as two rounded masses and seems to divide along with the other chromosomes. A count of an end view of one of the metaphase or stages of a late prophase of the

secondary spermatocyte division reveals two types of cells, one with eleven chromosomes and another with twelve. It may be possible that the chromosome which is lacking in the cell that shows a count of eleven is attached to one of the ordinary chromosomes as indicated by Miss King ('12).

Measurements of the head-lengths of the spermatozoa of Notophthalmus torosus show a distinct difference. A bimodal curve (text fig. D) may be readily plotted from the measurements obtained. Measurements of the head pieces of one hundred spermatozoa were taken and it was found that the spermatozoa with shorter head pieces are more common than those with longer head pieces. The ratio between the two types is approximately three to one. Due to the small number of observations made the ratio can only be considered approximate.

Text Figure D.

Graph showing dimorphism of the spermatozoa
of <u>Notophthalmus</u> <u>torosus</u>. Ordinates, length of
head-pieces of spermatozoa in mm. X 92;
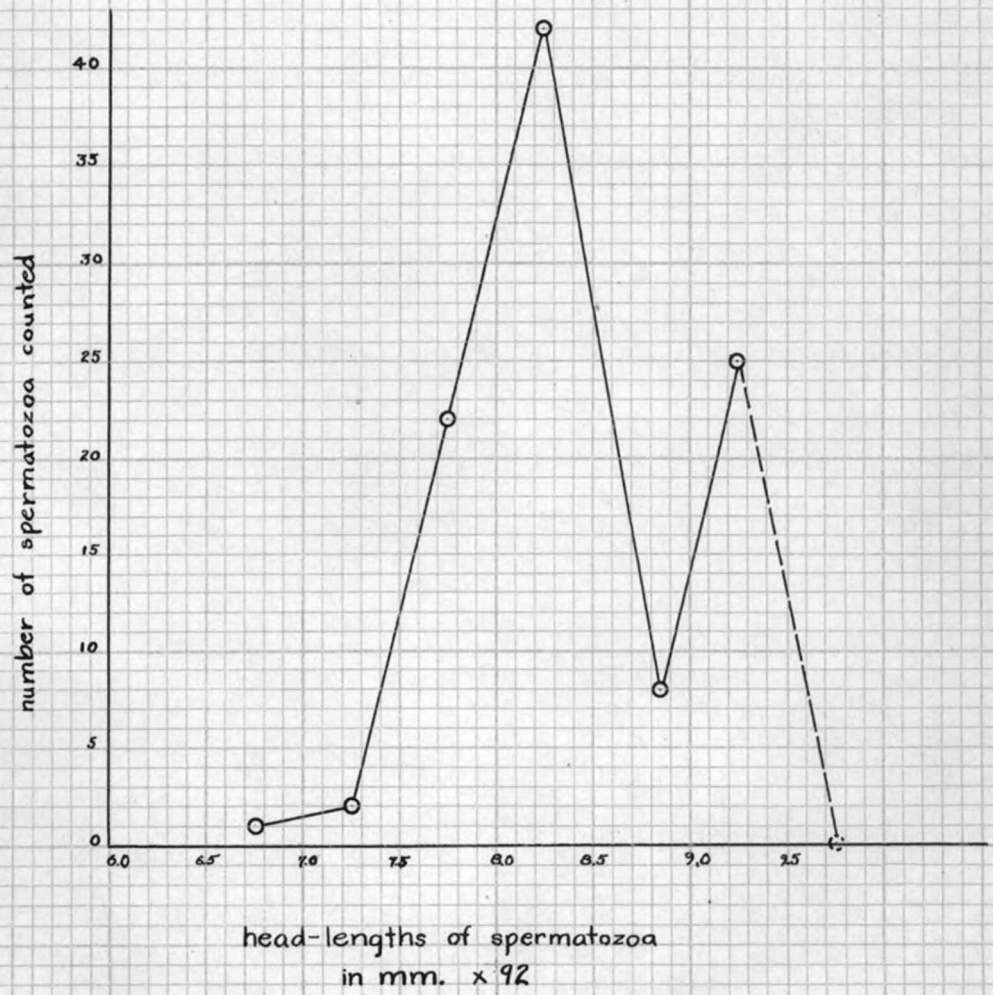Abscissae, number of spermatozoa counted.
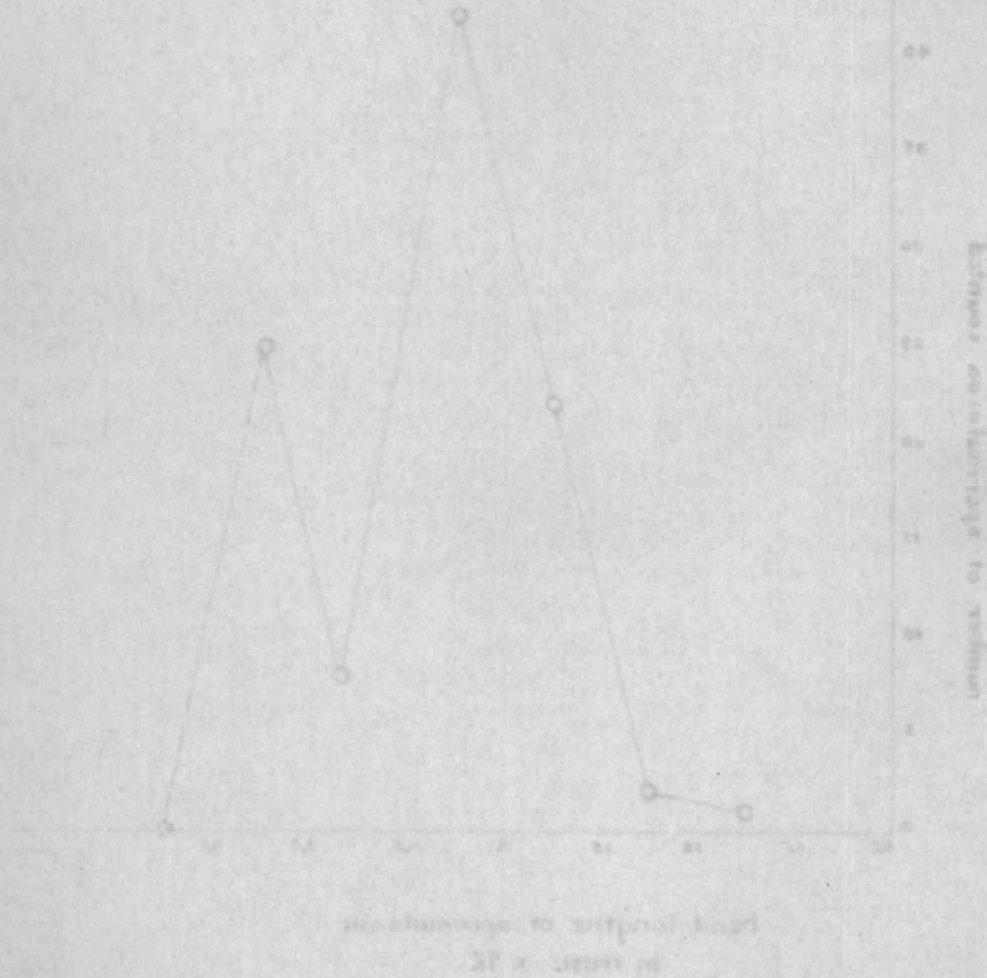
Figure D

SUMMARY AND CONCLUSIONS.

1. The male newt shows a remarkable seasonal variation in the soma. From early winter to the middle of the spring most of the males have a smooth skin. During the remain-der of the year both male and female have a roughened, tuberculated skin as well as a narrow tail.

2. Practically all somatic seasonal variations are lacking in the female newt. She has a roughened papillated skin and narrow tail throughout the entire year.

3. Males and females may be distinguished by their external appearances at all times of the year. This is not quite as apparent during the period in which the skin of the sexes is alike.

4. The responses of the animals to thigmotactic and photo-tactic stimuli are controlled to some extent by temperature.

5. There is a seasonal variation in the testes in both volume and internal processes. The maximum size of the testes is attained during early January; the minimum size occurs during the months of March and April.

6. Extrusion of the spermatozoa takes place during amplex-us. There are no spermatophores deposited.

7. The diploid number of chromosomes is twenty-four. The haploid number is eleven and twelve as shown by chromosome counts on the secondary spermatocytes.

8. A dimorphism exists in the spermatozoa, for there are

two types produced with respect to head-lengths; the

shorter type is of more common occurrence than the

longer type. The ratio between the two types is appro-

ximately three to one.

PROBLEMS SUGGESTED.

1. <u>The cloacal glands of the male</u>. There are two im-
   portant problems suggested: (a) the relation of
   the glands to the spermatozoa, and (b) the function
   of the "tongue-like elevation" in the cloacal cham-
   ber of the male.

2. <u>The origin of mucin</u>.

3. <u>The effects of the gonads</u>. The effects of extirpa-
   tion and also the effects of grafting testicular
   tissue into a sterile female and vice versa, are
   two closely related problems suggested by this topic.

4. <u>The effects of temperature, food, and injury</u>. Under
   this topic numerous problems could be considered.
   However, there are two which are outstanding; (a)
   the effects of temperature, food, and injury on the
   changes within the gonads, and (b) upon the breeding
   habits.

5. <u>Cytological problems</u>. Two important problems re-
   main; (a) a further study of the chromosome behavior
   during meiosis, and (b) the transformation of the
   spermatid into the spermatozoon.

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EXPLANATION OF PLATES.

All drawings with the exception of figure 1, were
outlined with the camera lucida. A 1.8 mm. oil immersion
objective and a 12× Periplan ocular were used in most in-
stances.

Plate 1.

Explanation of figures.

1. Ventral view of the male reproductive organs from
the right side of the body; c, corpus adiposum; g,
germinal epithelium; m, anterior half of mesonephros;
m', posterior half of mesonephros; o, termination of
Wolffian duct in the cloaca; t, lobe of the testis; w,
Wolffian duct. X 2.5.

2. Longitudinal section through lobe of testis; showing
succession of stages. A, anterior end; P, posterior end;
ps, primary spermatocytes; sg, spermatogonia; sp, sperma-
tids; sz, spermatozoa; ss, secondary spermatocytes; ef,
empty lobules. X 22.

3-6.Spermatogonia showing the succession of stages. To
the left are the earlier stages and to the right the
later stages. X 1200.

c

w

g

t

m

m'

o

1

sg

ps

ss

sp

A

sz

ef

P

2

3   4   5   6
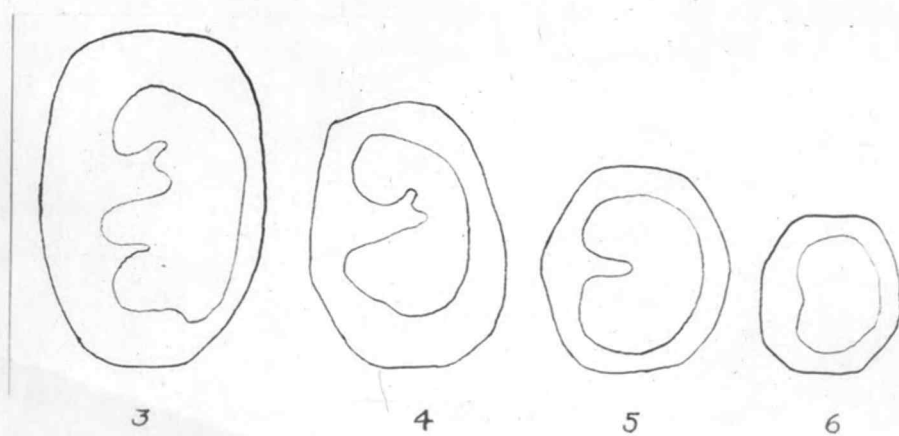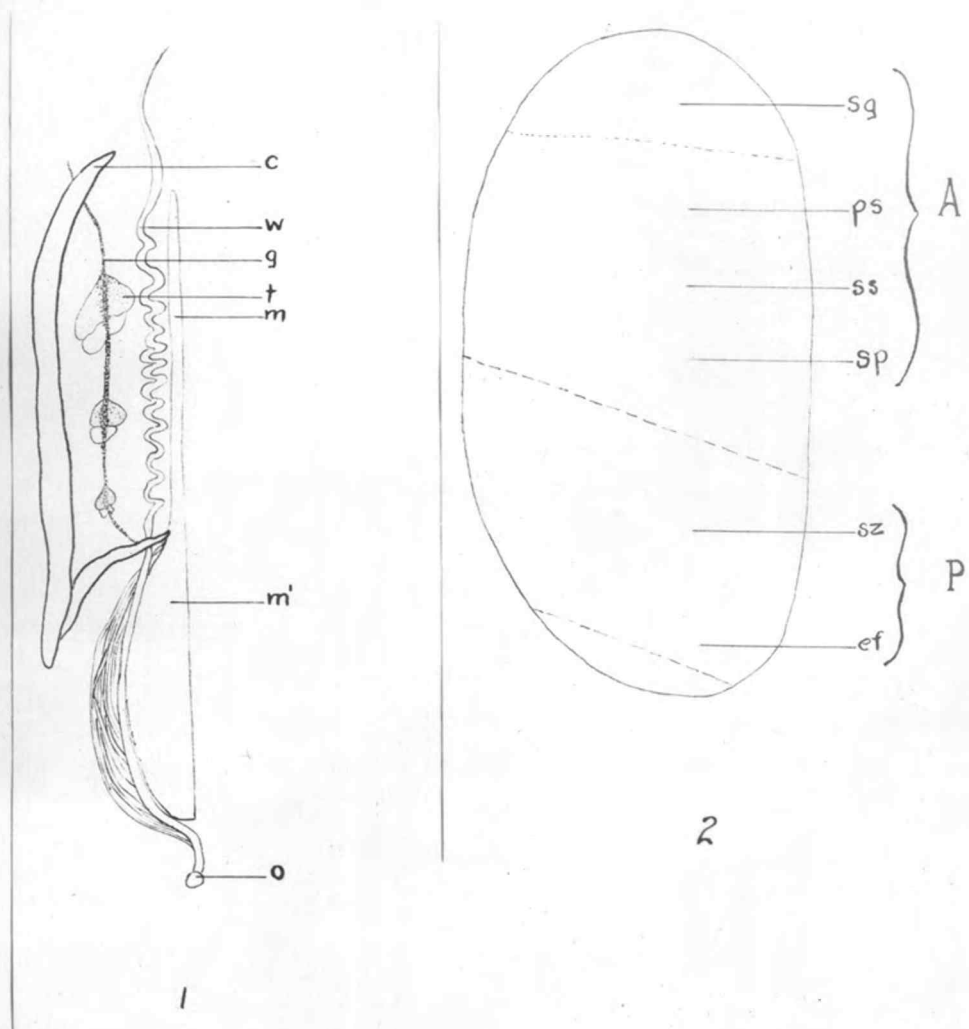
Plate   2.

Explanation of figures.

7. Resting condition of early spermatogonial stage.
    X 2000.

8. Polar view of the metaphase stage in an epithelial
    cell of the skin. The diploid number (24) of chro-
    mosomes is represented here. X 2000.

9. Early prophase stage of primary spermatocyte, show-
    ing 24 chromatin clumps. X 2000.

10. Leptotene bouquet stage primary spermatocyte. X
    2000.

11. Amphitene stage primary spermatocyte. Here the
    leptotene threads have come together side by side
    (parasynapsis) and are beginning to fuse in the
    region of the nucleus nearest the centrosome. X
    2000.
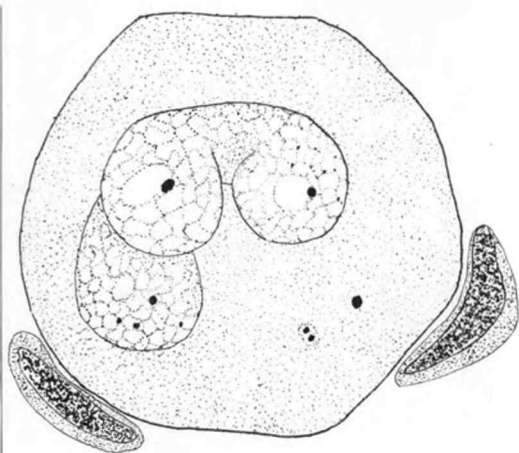
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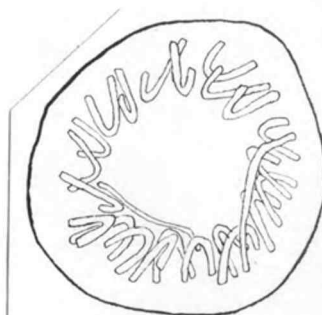
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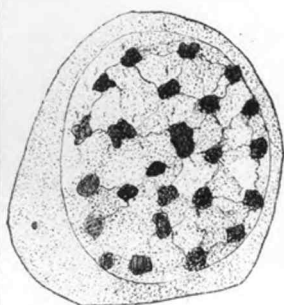
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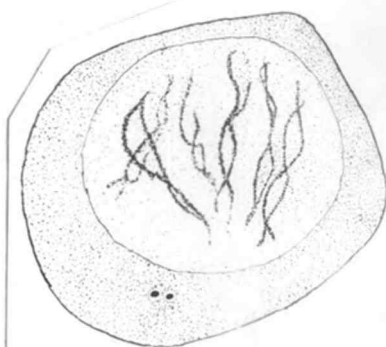Plate 3.

Explanation of figures.

12. Pachytene bouquet stage primary spermatocyte. The
     centrosome is found at one side of the free ends
     of the pachytene loops. X 2000.

13. Prediplotene stage primary spermatocyte, showing
     the manner in which the first split occurs. X 2000.

14. Diplotene stage primary spermatocyte. Here there
     is a twisting of the more condensed threads. X
     2000.

15. Late prophase, primary spermatocyte, showing some
     of the ring-like tetrads. X 1700.
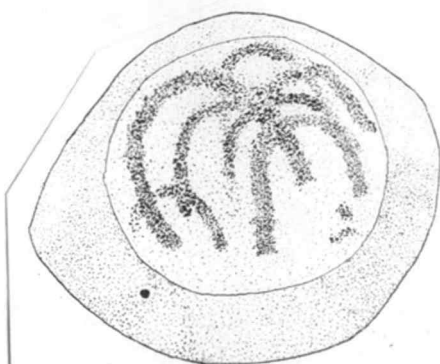
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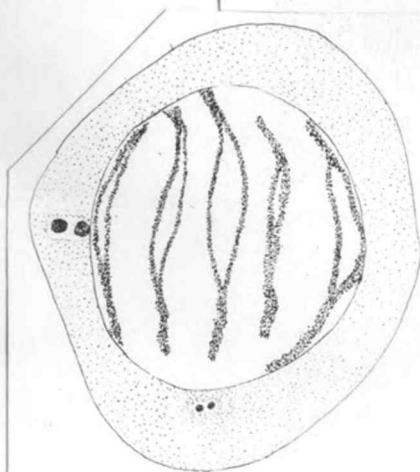
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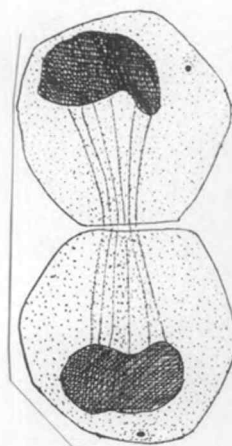## Plate 4.

Explanation of figures.

16. Metaphase stage of primary spermatocyte. X 1700.

17. Early anaphase stage of primary spermatocycte. X 2000.

18. Late telophase stage of primary spermatocycte. X 2000.

19.-20. Polar views of metaphase stages of secondary spermatocyte, showing eleven chromosomes. X 2000.

21. Polar view of metaphase of the secondard spermatocyte, showing twelve chromosomes. X 2000.

22. Anaphase, secondary spermatocyte stage. X 2000.
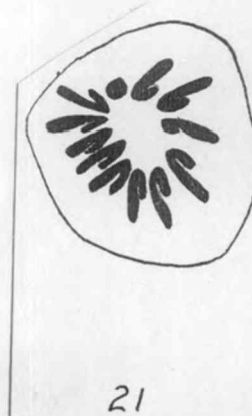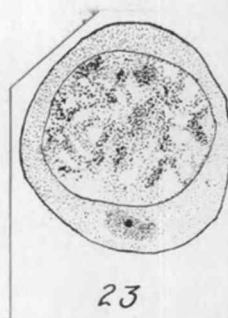
23. Resting of spermatid. X 2000.

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