THE REPRODUCTIVE SYSTEM OF THE MALE
NEOTENIC DICAMPTODON ENSATUS (ESCHSCHOLTZ)

by

NATHAN WOLF COHEN

A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

June 1956

APPROVED:

Associate Professor of Zoology

In Charge of Major

Chairman of Department of Zoology

Chairman of School Graduate Committee

Dean of Graduate School

Date thesis is presented ___August 15, 1955___

Typed by Betty M. Cohen

ACKNOWLEDGMENTS

It is with pleasure and gratitude that I acknowledge the constant encouragement and help of my major professor, Dr. Robert M. Storm, who has given freely of his time and support.

To Dr. Austin Pritchard goes thanks for his constant cooperation in making space available and for his generous support in helping provide necessary materials.

I should also like to thank Dr. Ernst J. Dornfeld who also has been most generous in providing materials and whose aid and advice on histological details has been most helpful.

I am deeply indebted to my wife, Betty, whose typing of this thesis represents but one small part of all of her selfless and generous endeavors cheerfully performed in aiding me in the completion of this study.

TABLE OF CONTENTS

THE REPRODUCTIVE SYSTEM OF THE MALE
NEOTENIC DICAMPTODON ENSATUS (ESCHSCHOLTZ)

INTRODUCTION

This study of the reproductive system of male neotenic
Dicamptodon was prompted by the fact that, although some features
of ecology, habits, and larval growth of Dicamptodon are known
(10, pp.49-55), no information is available about the histology
of its reproductive system. Neotenic males (figure 1), rather
than terrestrial adults, were used in this work because the former
are relatively more abundant. In addition, males were used in
this study rather than females because it was thought an investi-
gation of the histology of the reproductive system of the male
possibly would yield more information about the reproductive cycle
of this species.

Since the word "neotenic" has been used in several ways, its
meaning may be clarified at this point. As used herein, and as
defined by deBeer (1, p.52), neoteny refers " . . . to cases where
the adult animal retains larval characters." The outstanding larval
characteristics of neotenic Dicamptodon are the retention of
external gills and the continuation of aquatic habitat.

The major part of this investigation is a histological
description of the reproductive system. In order to give clarity
to the orientation and spatial relationships of the structures

involved, a description of the gross anatomy of the tissues and organs precedes the histological description.

Field and laboratory work have resulted in some ecological and other additional information which follows the presentation of the histological studies.

Preparation of Histological Material

Some animals were killed within hours after they were collected, others were maintained in the laboratory for several months or longer before they were used.

All animals intended for histological study were perfused with Bouin's fixative through the heart, and the organs were removed and put into Bouin's.

Some of the tissues were dehydrated in dioxan and subsequently infiltrated with paraffin. Large organs or tissues were dehydrated and cleared in aniline oil, then infiltrated with paraffin. Hansen's paraffin mixture (5, p.646) was used for infiltrating and embedding.

Most tissues were sectioned serially at 5 μ, some at 8 μ or 10 μ, depending upon the tissue. Spaced serials, when mounted, represented a distance of 0.25 mm. to 0.5 mm. from one part of an organ to the next, depending upon the thickness and depth of the section.

Masson's trichrome stain (9, p.45) was used on all tissues. This stain gives a rather sharp differentiation of the following: mucus and connective tissues stain green; muscle, cytoplasm and epithelium stain shades of pink; nuclei stain black; and blood

cells stain orange.

Collection of Dicamptodon

Neotenic Dicamptodon were taken by two different methods.
One method used, which was originally shown to me by Dr. Robert M.
Storm, was to first chum the water of a clear-running stream with
a well-battered piece of fresh fish tied to a rock. As soon as a
Dicamptodon, attracted by the submerged bait, left its place of
concealment and became visible as it slowly made its way upstream
to the piece of fish, another small piece of fish tied to a
weighted fishing line was gently lowered into the water so that it
came to rest on the stream bottom about one foot or closer in
front of the advancing animal. In most instances the Dicamptodon
would seize the bait vigorously and, when it met resistance in
trying to pull it away from the spot, would begin to spin violently
around in the water like a spinning lure. At this moment the line
would be raised slowly and evenly, and a hand-net used to ensure
its capture.

The other method was to bait a funnel trap with fresh fish,
submerge it in the water and return within the next few days to
retrieve the trapped animals. This latter method was seldom used
successfully in a stream, but was successful when used in a pond.

GROSS ANATOMY OF THE CLOACA OF MALE AND FEMALE
NEOTENIC DICAMPTODON

The cloaca may be considered as consisting of two parts; an enlarged narrow caudal chamber opening ventrally to the exterior by way of the cloacal lips, and a complexly-shaped cephalic tubular portion which connects the cloacal chamber to the rectum. These two regions of the cloaca will be discussed separately. In addition, even though the histology of the reproductive system of the female has not been included in this study, the gross anatomy of the female cloaca is herein described and compared with the male cloaca. This has been done in order to aid other workers who may desire to distinguish the sexes of Dicamptodon by using only external features.

External Appearance of the Cloacal Region

The external features of the cloaca of male and female are similar (figures 2 and 3, drawn from living animals). However, certain distinguishing features are present and will be discussed below. The cloacal aperture is simply a longitudinal slit bordered by two inconspicuous lips which are distinguished only by a narrow unpigmented ring surrounding the slit. During the breeding season the cloacal lips of both sexes may gape slightly. In the male, but not in the female, a papilla is present which projects caudad from the anterior dorsal end of the external cloacal opening into the slit near the surface of the opening. This papilla, referred to as the cloacal papilla and normally not seen unless the cloacal lips

are pulled apart slightly, is one of the distinguishing features
of the male.

Walls of the Cloacal Aperture. In both sexes the sides of the
walls of the external orifice are divided into an anterior and a
posterior part. In the breeding condition, males may be easily
identified by the series of fine ridges which occupy the anterior
portion of the cloacal opening. These ridges, which are unpigmented,
uniformly fine, and parallel, slant in a dorso-anterior, ventro-
caudad direction. They are deep red in color due to considerable
vascularization. The slant of these ridges is greatest at the
posterior end and gradually diminishes toward the cloacal papilla.

Females also have ridges on the orifice walls but the con-
figuration of the ridges shows definite structural differences
between the sexes. The ridges in the female occupy a greater area
of the cloacal wall, are wider and shorter, not as parallel to
each other, and not as close together as they are in the male.
As a result of this, this portion of the wall is not as deep red
as the corresponding part of the male.

The ventral and lateral surfaces of the male cloacal papilla
are also thrown into a series of ridges like those of its adjacent
cloacal wall. These ridges are seen in transverse section in
figures 11 - 13.

The posterior half of the cloacal wall in both sexes is
relatively smooth and has a deposition of melanophores whose
greatest concentration is near the ventral, posterior part of the

wall. Males have a greater abundance of these melanophores which also differ in distribution from those in the female (figure 2). Although melanophore deposition in the individuals studied is somewhat greater in males than in females, as shown by stippling in figure 2, melanophore development may be variable, depending upon seasonal activity.

In the male, the roof of the cloaca opposite the cloacal papilla has a deep narrow depression, which becomes deeper and forms lateral extensions at the caudal end of the cloacal orifice. This dorsal furrow is best seen in transverse sections (figures 12 - 16). A more detailed treatment of this portion of the cloaca will be discussed under the histology of the cloacal wall.

Internal Topography of the Cloacal Cavity

Dissection of four specimens and histological confirmation show that the urogenital ducts in the male enter the cloacal cavity dorso-laterally very near the cephalic end of the tubular portion. The entry of each duct into the cavity is marked by a slight pit-like depression surrounding a small papilla through which the urinary and reproductive material passes. A small well-defined groove runs from each urogenital papilla meso-cephalically, and these join at the cephalic neck of the cloacal chamber near the opening of the urinary bladder. The urinary bladder opens medially into the ventral side of the cloaca slightly anterior to the urogenital duct entrances, so that any

urinary fluid entering the bladder has to flow first into the cloacal cavity.

In the relaxed, undistended condition, the mucosal wall of the tubular portion of the cloaca is thrown into high, thin parallel ridges which at the caudal end of the cavity become appressed and close off this region from the external slit. These ridges continue, however, as the ridges of the cloacal aperture. In the specimen from which the camera lucida drawings (figures 4 - 19) were made, the cloacal walls were not pressed together in the histological preparations.

Dissection of three specimens shows that in the female cloaca, the main distinguishing features are that the two oviducal papillae, which correspond in location with the urogenital papillae of the male, are considerably larger than those of the latter, and protrude about 1 to 2 mm. into the cloacal lumen. The female oviducal papillae are also surrounded by a pit-like depression into which the urinary ducts open medio-laterally.

Unlike the plethodontids and some other families of urodeles (8, p.286), melanophore deposition in Dicamptodon is absent in the roof of the cloaca; and in the underlying connective tissue pigmentation is scarce, which is somewhat contrary to the situation in Necturus (3, p.450).

THE UROGENITAL SYSTEM OF THE MALE NEOTENIC DICAMPTODON

Gross Anatomy of the Male Urogenital System

For the sake of clarity, a brief description of the structural
relationships of the urogenital system and the terms used for the
various structures will be given. There is some disagreement among
various authors regarding the terminology used to describe the
urogenital apparatus. The correct designation for the various
units of the urogenital system would ultimately depend largely,
of course, upon a thorough embryological study. Inasmuch as no
such study has been made upon Dicamptodon, one can only try to be
judicious in the choice of terms adopted. The description of the
urodele urogenital system as given by Hyman (6, pp.394-395) best
fits the case for Dicamptodon and will be used here for most of
the structures. Although the genital and urinary kidneys, particu-
larly the latter, have not been studied in this investigation
except as they are specifically related to the reproductive system,
I freely use the term urogenital system throughout this paper.

Testes and Ducts. The testes of mature Dicamptodon are paired
structures nearly equal in size and do not exhibit the "multiple
testes" condition which is found in many other families of salaman-
ders, particularly in the plethodontids. In this respect they are
similar to the testes of Necturus as figured by Chase (2, p.521).

The following description is of one of the testes from a
mature neotenic individual which measured 160 mm. in snout-vent

length. The testis is 46 mm. in total length, 6 mm. wide and 2 mm. in depth at its greatest width. Blunt at its posterior end, which is adjacent to the anterior portion of the rectum, the structure begins to taper rather sharply at a region 25 mm. forward of the posterior end and then continues forward as a thick band 2 - 3 mm. wide in cross section until it terminates in the region of the cardiac portion of the stomach.

From my studies it was observed that the testes are enclosed by a thin connective tissue capsule 6 μ - 12 μ wide which contains typical spindle-shaped nuclei. The testicular mass is made up of seminiferous tubules bound together by thin strands of connective tissue. Only certain groups of tubules, varying in number from one to all of those occupying one-half or more of the cross-sectional area of the testes, produce sperm at any one time. These groups, as well as tubules inactive in spermatogenesis, constitute the lobules (figure 20).

In each testis a testicular network of fine ducts carry the sperm from the active tubules to the mesial edge of the testis where they leave that organ through another series of ducts, the efferent ductules, which are variable in number from one to five. The testicular network, carrying the sperm to the efferent ductules, may be divided into intralobular and interlobular ducts (figure 21). An intralobular duct receives sperm from a seminiferous tubule and several of these ducts converge to form the larger interlobular ducts which convey the germinal products to the efferent ductules.

Several interlobular ducts may merge together before reaching the efferent ductules.

The efferent ductules pass through the mesorchium to the opisthonephros (figure 22), which in the male constitutes the epididymis (6, p.395), genital kidney (2, p.502), or sexual kidney (4, p.286). This portion of the opisthonephros in Dicamptodon apparently does not serve any urinary function, since no nephrostomal structures are present. Chase (2, pp.503-504) found the same condition to exist in Necturus, and states further that Spengel[1] showed that the nephrostomes degenerate in the genital kidney of all adult males of urodeles. In so far as I was unable to locate nephrostomes in the genital kidney of adult males examined, neotenic Dicamptodon is no exception to this. The sperm-conveying tubules within the genital kidney have been termed epididymal tubules (6, p.395). According to Chase (2, p.503) the renal tubules of the genital kidney, in Necturus, are connected, at their renal corpuscles, with the tubes of the epididymis as mentioned above. Although I could not demonstrate in the spaced serial sections the actual connection between the epididymal tubules and the renal tubules, the same situation must apply in Dicamptodon, since sperm have been found in all the ducts mentioned.

According to Chase (2, p.503), who described the pathway for

[1]Spengel, J. W. 1876. Das Urogenital system der Amphibien. Arbeit. Zoöl. Zoöt. Inst., Würzburg, Bd. 3, S.42-114, Taf. 1-4.

Necturus sperm, the sperm, after having entered the epididymis, then enter the collecting duct of the kidney from the efferent ductules and are carried successively to the renal corpuscle thence into the proximal convoluted tubule, through the distal convoluted tubule and into a junctional tubule (8 to 12 in number for each testes in Dicamptodon) which empties into the Wolffian duct.

Wolffian Duct. In pre-mating, breeding Dicamptodon males, the Wolffian duct is seen to be nearly circular or oval in cross section and filled completely with sperm suspended in a liquid medium (figure 23). Anteriorly the Wolffian duct is much convoluted, but straightens out anterior to the urinary kidney and runs a course parallel with the lateral edge of the kidney down to the urogenital papilla. Since the Wolffian duct acts to store spermatic fluid, it has been aptly called a seminal vesicle.

Sperm are also found in the urogenital ducts, which in Dicamp- todon arise from the lateral wall of the kidney as a series of 8 to 11 tubes of gradually decreasing length; most of these tubules pursue a more or less parallel course around the ventro-lateral aspect of the kidney, until they converge toward the cloacal wall at the site of the urogenital papilla or first enter the Wolffian duct and then pass into the urogenital papilla (figure 24).

Some of these urogenital ducts may merge into a larger duct immediately before emptying into the orifice of the urogenital papilla, but many of them enter this orifice independently. A close

study of this region also showed that several of the urogenital ducts emptied into the Wolffian duct a short distance anterior to the base of the urogenital papilla.

In addition to having observed sperm in the urogenital ducts, it may also be noted here that sperm were observed in the collecting tubules (figure 22) and in the distal convoluted tubules of the Dicamptodon urinary kidney (figure 25). Chase (2, p.508) noted that in Necturus sperm were found in the distal convoluted tubules of the genital kidney, and explained their presence in those tubules as a result of their having entered from the Wolffian duct either by their own activity or due to muscular contractions of the Wolffian duct.

The presence of sperm within the urinary kidney of Dicamptodon may possibly be due to the same causes which Chase ascribes for the similar condition in the genital kidney of Necturus.

Histology of Active Testes and Ducts

The following description is based upon a study of the testes of three mature neotenic males which were immediately killed following collection during the month of February 1955.

Testes. Although not showing the "multiple testes" structure, the testes of Dicamptodon are characterized, as they are in Taricha, by regions which contain mature spermatozoa and by regions which contain primary and secondary spermatocytes and spermatogonia. Both of these regions, the one containing mature spermatozoa and the

other made up of the aforementioned tubules, as previously stated, may be called lobules.

The appearance of the testes, based on the observations above and on a study of inactive testes (discussed in the last part of this section), is morphologically and histologically correlated with seasonal changes, and the external appearance of these gonads is correspondingly correlated with internal changes. The mature tubules of the testes in the following discussion are in the process of evacuating their sperm into the efferent ducts and the Wolffian ducts are filled with sperm. In figure 26 the last of the sperm of a tubule can be seen about to enter one of the intralobular ducts.

According to Miller and Robbins (7, p.442) shortly after evacuation of the mature tubules in Taricha torosa, the connective tissue cells surrounding each tubule form a ring of cuboidal lipid-filled cells surrounding the Sertoli elements, and at the same time the Sertoli tissue develops cell boundaries and becomes an inner ring of lipid-filled cells. Since lipid material was dissolved away in the histologic technique used, the presence of lipid material cannot be definitely demonstrated, but the coarse, reticular nature of the Sertoli cell cytoplasm seems to justify the belief that these cells likewise become filled with this material. The inward movement of the Sertoli cells and their encirclement by a layer of cuboidal cells usually takes place just as the sperm are being evacuated or immediately after evacuation. In some cases the internal tubular changes begin to take place even before the tubules are

evacuated. The sequence about to be described for Dicamptodon

was first worked out in Taricha by Miller and Robbins (7, p.422),

and follows fairly closely the changes observed in the latter

species.

During evacuation or just after, the Sertoli cells develop

cell membranes as well as a collagenous internal limiting membrane

which separates the Sertoli elements from the connective tissue-

derived elements (figure 26). Since the Sertoli cells and

connective tissue cells are very similar in appearance, both types

having a wrinkled or smooth nucleus containing coarse chromatin

granules, the development of the internal limiting membrane enables

one to easily distinguish between the two types of cells. Accord-

ing to Miller and Robbins (7, p.422) this double-layered structure

is of dual origin and becomes the so-called glandular, epithelioid,

or interstitial tissue.

In Dicamptodon the mature tubule, which ranges in size between

100 - 122 µ in diameter, is, of course, devoid of the connective

tissue cells. For some time preceding evacuation, the tubule is

outlined by a ring of low cuboidal cells, usually in a single layer,

and bounded internally by a very thin limiting membrane which is of

approximately the same thickness as the external limiting membrane.

As the Sertoli cells move toward the center of the tubule, the

internal limiting membrane becomes thickened. The nucleus of these

Sertoli cells in the unevacuated tubule is flattened to oval in

shape and takes on a more spherical shape as movement inward takes

place. I could find no interstitial cells morphologically homologous to those in mammals.

The mature sperm within the tubules are grouped in loose whorls. The nucleus-containing portion or head of each sperm is very dark in color due to its chromophilic nature; this sharply separates it from the tail portion which takes the red stain.

Many of the Sertoli cells in evacuated tubules appear to be undergoing gradual dissolution, corresponding to the changes which take place in Taricha torosa. In the mature testes of Dicamptodon the glandular or interstitial tissue appears much like the Sertoli and connective tissue cells in evacuated tubules. As stated above, Miller and Robbins are of the opinion that the interstitial tissue of Taricha torosa is of dual origin but point out that the amount contributed by Sertoli and connective tissue elements may vary among different species of urodeles.

Ducts of Testes. The histology of the intralobular and interlobular ducts are similar, as shown in figure 26. The sperm leaving the seminiferous tubules enter the intralobular duct through a narrow opening which gradually widens to a diameter of from 75 - 90 μ. The walls of the intralobular ducts are composed of an outer collagenous connective tissue, 10 - 15 μ in thickness, and lined internally by low cuboidal, unciliated cells 10 - 15 μ across having spheroidal or ovoid nuclei and finely granular, undifferentiated cytoplasm. The interlobular ducts, receiving the contents of several intralobular ducts, vary from slightly larger in diameter

up to 420 μ or more across. Their structure is similar to that
described for the intralobular ducts, but their connective tissue
covering is considerably thicker. No pigment bodies have been
observed within the walls of any of these ducts, but a few
melanophores were observed investing testicular blood vessels.

Efferent Ducts. The interlobular ducts empty into the efferent
ducts near the ventro-mesial surface of the testis (figure 27). The
efferent ducts, which vary in number from one to five, are com-
paratively large, measuring up to 0.5 mm. in diameter, but usually
less. They have the same structural features as the preceding
ducts, except for a denser connective tissue covering. The
connective tissue, although variable in thickness and presumably
elastic, may be as much as 50 μ in thickness. If one of these
ducts is greatly distended with spermatic fluid, the nuclei of
the cells lining the lumen project into it under a thin, unciliated
cellular membrane.

Sperm and Renal Ducts of the Epididymis. The efferent ducts
narrow as they enter the epididymis and are easily distinguished
from the renal tubules primarily by the fact that the renal tubules
are 3 - 5 times larger in size than the sperm ducts and are ciliated,
whereas the epididymal tubes are unciliated (figure 22). Within
the epididymis the sperm ducts do not greatly exceed 90 μ in
diameter, whereas the renal tubes commonly exceed 250 μ in diameter.
The sperm ducts, which have a comparatively heavier connective
tissue wall than the kidney tubules, are lined by cuboidal cells

containing large nuclei filled with a very coarse granular chromatin material. All of the tubules within the epididymis are surrounded by a sheath of spindle-shaped connective tissue cells. The supporting tissue of this structure is composed of a reticulum of connective tissue fibers among which are numerous leucocytes and capillaries.

The cells of the proximal convoluted renal tubules are more commonly low columnar rather than cuboidal, containing ovoid nuclei filled with coarse chromatin material and surrounded by moderately granular cytoplasm. Two types of cilia are present within these renal tubules: short, independent cilia projecting $4.5\ \mu$ into the lumen, whose basal and distal ends can be distinguished; and very long cilia, some of which appear to be gathered into long tufts. These long cilia may exceed $60\ \mu$ in length. The short cilia appear to be rather uniformly distributed, but the long cilia occur in definite, irregularly distributed groups within the lumen.

The distal convoluted tubules are considerably larger than the proximal tubules, being in the nieghborhood of $240\ \mu$ in cross section in the region adjacent to the main tissue mass of the epididymis. The distal convoluted tubules are nearly always filled with sperm in a breeding animal. This tubule, which is probably distensible, is lined with low cuboidal, unciliated cells, whose nuclei are flattened ovoid to spheroid. The distal convoluted tubule joins the junctional tubules which run to the

Wolffian duct.

In figure 28 can be seen the site where the junctional tubule empties into the Wolffian duct. The histology of the lining cells of the junctional tubules and the Wolffian duct, where the former empties into it in the anterior region of the genital kidney, is quite similar and the details given below for the lining cells of the Wolffian duct apply equally to those of the junctional tubule.

The Wolffian Duct. The anterior portion of the Wolffian duct is variable in the thickness of the wall and height of the lining epithelial cells, depending upon the distension caused by the mass of sperm.

The Wolffian duct of the male is composed of four types of tissues. The peritoneal covering overlaps a thin layer of connective tissue which is interspersed with another very thin layer of spindle-shaped smooth muscle cells. The innermost layer is the epithelial lining of the duct. The three inner layers differ histologically in the anterior and posterior regions of the Wolffian duct and will be discussed further below. The anterior portion of the Wolffian duct, in particular, probably serves as a seminal vesicle, since it contains very little tissue or cells that are suggestive of secretory activity.

In the breeding male, the anterior-most portion of the Wolffian duct often exceeds 0.5 mm. in diameter, and has a comparatively thin wall, both the outer covering of connective tissue as well as the epithelial lining being reduced in size

compared with that part of the duct adjacent to the pelvic kidney.
In the region adjacent to the main portion of the genital kidney,
the connective tissue and smooth muscle cells in the outer wall of
the Wolffian duct are compact, the wall averaging about 25 μ in
thickness. Blood supply is relatively sparse. The cells of the
lining epithelium are cuboidal to low columnar, ranging from less
than 18 μ to 45 μ in depth. The free cell boundaries are usually
indistinct and the cytoplasm is filled with a very light green-
staining granular mass. In some regions, masses of glandular
material are in the stage of dissolution on the free surface of
the cells and beginning to enter into the mass of sperm and light
green-staining material already present within the lumen of the
duct. The nuclei of these cells rest on the basement membrane
and are broadly ovoid in shape, are from 5 - 15 μ in diameter
and are filled with a coarse chromophilic material. Glandular
activity appears comparatively moderate in this region of the
Wolffian duct.

The Wolffian duct in the neighborhood of the pelvic kidney
and cloaca (figure 29) differs in several respects from its more
anterior portion, but the histological transition between the
two regions is gradual. The posterior Wolffian duct is of about
the same diameter as the anterior portion, and is also filled with
sperm and fluid, but the duct diameter is quite variable. The
duct here is elongated in a plane paralleling the contours of
that part of the pelvic kidney to which it lies adjacent, presenting

an oval-shaped structure in cross section. The wall of the duct,
including the peritoneal layer, and connective tissue and muscle
layers, ranges in thickness from 80 - 115 µ, and is not compact
as it is in the anterior part of the duct. There are many capil-
laries and other spaces within the connective tissue. The layer
of smooth muscle is more evident, occupying a more prominent portion
of the connective tissue mass.

The lining epithelium of the posterior Wolffian duct of
Dicamptodon is made up of very active glandular, columnar cells.
The majority of these cells are 100 µ or more in length. The
nuclei are oblong in the long direction of the cell, rest on the
basement membrane, are 15 - 20 µ in length and contain large
discrete masses of irregular-shaped chromatin material. The active
secretory regions of these cells is most apparent just above the
nucleus, where fairly large droplets of orange-staining secretions
may be observed. In other sections these orange-stained droplets
may be seen throughout the whole cell mass. The rest of the cyto-
plasm is filled with equally large droplets which, however, take
a light green stain. Numerous cells at their free surface are in
the process of evacuating their glandular products into the lumen
of the duct. Free nuclei at the superficial surface of the lining
cells were noted here as they were in the anterior portion of the
duct. These free nuclei occupy the same position as they do more
anteriorly in the duct, but in some instances a fine, thin
connection may project into the intercellular space of two adjacent

cells on whose surface the nucleus lies. Chase (2, p.469) also noted free spindle-shaped nuclei between the glandular cells, but he stated that he could not detect under high magnification any cytoplasm belonging to the free nuclei. Some of these nuclei are within the intracellular spaces (figure 29), thus being suggestive of the interpretation that these free nuclei originally belonged to glandular cells and are now in the process of being removed, their cytoplasm having been dissoluted earlier. However, no free nuclei have been observed within the lumen of the Wolffian ducts.

Histology of the Inactive Testes and Ducts

The following description is based upon the testis of a mature neotenic Dicamptodon which had been held in captivity for 4 months and was then killed on February 23, 1954.

Testes and Ducts. The testis is composed of what appears to be unorganized interstitial material, loosely made up of connective tissue fibers and cells and Sertoli cells, and bears no organized histological resemblance whatsoever to active testicular tissue. This condition is suggestive of the degenerated condition of testes of Taricha torosa after the termination of the breeding season (7, p.422). In this Dicamptodon testis, degeneration seems complete (figure 30), extending even to the intra- and interlobular testicular ductules which appear to be completely absent.

The efferent ducts which are embedded in connective tissue range in diameter between 64 - 168 µ, and are bounded by a limiting

membrane 1 - 2 μ in thickness. The efferent ducts are lined by a single row (in most cases) of cells ranging in size between 15 - 35 μ in their greatest cross section, varying in shape between low cuboidal to columnar. The nuclei, which lie close to the basement membrane, vary in shape from spheroid to an elongated ovoid and are from 7 - 13 μ in diameter.

Wolffian Duct. The Wolffian ducts in the non-breeding male are completely devoid of sperm, and vary slightly in size, along their tortuously coiled path, from 800 - 850 μ in diameter.

The wall of the Wolffian duct adjacent to the testes in a non-breeding male is composed of a heavy layer of fibrous connective tissue, 50 - 100 μ in thickness, containing numerous connective tissue cell nuclei evenly distributed throughout. This wall is fairly well vascularized, the capillaries being quite evenly distributed, about 60 μ apart, near the external limiting membrane. The lumen of this duct is surrounded by a layer of pseudostratified cells. The cellular membranes are generally difficult to distinguish; however, a few cells whose membranes were distinguishable, measured about 100 μ in length. The basal nuclei appear to be resting upon the basement membrane and form a continuous ring. The nuclei between the inner- and outer-most cells of this layer are irregular in their distribution. Some of the free nuclei appear to be actually on the free surface of the internal limiting cell membrane. All of these nuclei contain coarse chromatin material and their cytoplasm is coarsely granular. The internal limiting membrane has

a thin deposit of material on its surface. This material appears
to be debris and of the same nature as that seen in the center of
the lumen.

Although it is not possible to describe the complete seasonal
changes in the testes since representative material was not avail-
able at different seasons of the year, a comparison between the
active and inactive testes described above is suggestive of the
male gonadal cycle. Active and inactive testes were removed from
Dicamptodon in February of 1954 and 1955.

Admittedly, the quantity of material examined is insufficient
to permit definite conclusions regarding the seasonal testicular
activity, but the histological nature of the active and inactive
testes that have been examined, does suggest that the male repro-
ductive cycle in Dicamptodon is similar to that which occurs in
Taricha. In Salamandra salamandra, Francis (4, p.5) states that
sperm developed during one breeding season are not transferred to
the female during the same breeding season, but are stored in the
receptaculum seminis and remain there through the winter, fulfilling
their ultimate purpose in the following May or June.

THE CLOACAL GLAND COMPLEX OF THE NEOTENIC MALE DICAMPTODON

The masses of glandular tissue adjacent to and surrounding the cloacal walls of male Dicamptodon are referred to collectively as the cloacal gland complex. The internal configuration of the cloacal walls adds further to the complication of working out the relationships of the several types of glandular tissue.

It is believed that some of the glandular tubules of the cloacal gland complex in male salamanders produce the spermatophore; a small, firm, gelatinous blob of varying shape in different species, in which the sperm are embedded (8, p.283). It is to be expected, then, that the glandular tubules, like the rest of the reproductive apparatus of Dicamptodon, vary histologically from one breeding season to the next. It can only be assumed that Dicamptodon produces spermatophores, since nothing is known of its mating behavior. Which glands participate in spermatophore formation may be partly deduced by determining into which regions of the cloaca the various glands empty their products.

The greatest difficulty in distinguishing the tubules of the different groups of glands has been well stated by Dawson (3, p.453):

Owing to the great variety of secretory phases exhibited by the different tubules, it is not always easy to determine with certainty whether the tubules under consideration are of an entirely different character or are merely different phases of activity of the same kind of tubule.

This difficulty applies particularly to the different groups of pelvic tubules.

Although cloacal glands are present in all ambystomids, salamandrids, and primitive plethodontids (8, p.284), the three different types of glands making up the cloacal glandular mass are not present in equal proportion in all those families named above; and, indeed, some families of salamanders may be lacking in one or more of the three types.

Gross Anatomy of the Cloacal Gland Complex

Dawson (3, pp.447-465) described the anatomy of the cloacal gland complex of the male Necturus. A study of the various glands which constitute the cloacal gland complex of Dicamptodon reveals that, in most instances, they correspond fairly closely in spatial relationships with those of Necturus. The description below is that of a mature neotenic male.

The large glandular mass about the cloaca is composed of great numbers of tubular glands, which can be differentiated into several distinct groups. Distinction of these different glandular unities is based upon the histological nature of the cellular components of the glandular tubules and their secretions. At least three distinct types of tubules have been distinguished. Organized groups of these tubules are known as the cloacal, pelvic and abdominal glands. In the following discussion each of these glands will be treated as separate entities, in which the gross anatomy, spatial relationships and histology will be presented in that order.

A perusal of figures 4 - 19 will help in understanding the

distribution and relationships of the various glands. These drawings were done with the aid of a camera lucida and represent more or less evenly spaced transverse sections from the anterior origin of the cloacal cavity through its caudal termination. In these drawings some representative tubules of each gland are outlined in their glandular masses to show their respective size and orientation. Because of their relatively large size, a greater number, but not all, of the abdominal gland tubules have been drawn. In most cases the tubules of one gland are interspersed, to varying depths, with tubules of an adjacent gland. Areas containing these mixed tubules are depicted in the drawings by overlapping of the lined and solid portions.

Where there appears to be more or less definite boundaries between two types of tubules, or where interspersion is largely confined within narrow regions, a line has been drawn to indicate the boundaries or regions of interspersion. In only one case do these lines represent actual connective tissue septa, this exception being the septa which, on either side of the cloaca, separate the anterior portion of the anterior pelvic glands from other glands (figures 4 - 6). In all other cases the drawn lines only indicate the limits of the different glands, with no implied presence of connective tissue septa. Furthermore, the nearly uniform heavy outlines of the walls of the cloacal chamber do not bear any correlation with the thickness of the wall in the different sections.

Anatomy of the Cloacal Glands

The orientation of the main body of tubules of the cloacal gland may be seen in figure 23 which is a frontal section of the cloacal region, and corresponds approximately with the level of the tip of the cloacal papilla in figure 12. Most of the cloacal tubules are slightly coiled and oriented so that they empty into the cloacal cavity through the edges of the ridges of either the cloacal wall or the cloacal papilla; less commonly they have been observed to open into the smooth posterior portion of the walls of the external cavity of the cloaca. These tubules terminate in blind ends; most of them, as far as I have been able to determine, being at the periphery of the glandular mass.

The major portion of the cloacal gland tubules lie caudad to the main axis of the pelvic girdle, but a small part of the gland extends anteriorly beyond the caudal edge of the ischium and projects forward (not shown in this section) just beyond the caudal end of the pelvic kidney, along the ventro-lateral edge of that organ. In this region the Wolffian ducts have not yet emptied into the cloaca.

The cloacal gland tubules in this anterior region are paired cephalic extensions of the main glandular mass and lie just dorsal to the cartilaginous ischium, and dorso-laterally to the urinary bladder (figure 4). Posteriorly, both of these extensions expand in a dorso-ventral plane so that they occupy a narrow region on either side of the bladder where this organ and the Wolffian ducts

empty into the cloaca.

The lateral walls of the cloaca in this region are heavily invested with a thick musculature, and the cloacal glands are further separated from the cloaca by connective tissue septa (figure 6, not labeled). Other glandular masses (see pelvic glands) make their appearance in this region.

More posteriorly, the lateral extensions of the cloacal gland meet in the medial, ventral region between the cloaca and the posterior medial projection of the ischium (not drawn, but region between figure 6 and figure 7). The previously-mentioned connective-tissue septa are no longer present, and the tubules of these glands are beginning to penetrate the investing cloacal musculature which is not as compact here as it is more anteriorly. The cloacal tissues are well-vascularized, receiving their blood from the large caudal artery which lies between the ventral trough of the cloaca and the medial cloacal glands.

Another pair of very short projections of the cloacal gland now make their appearance, lying between the ventral surface of the above-mentioned ischium projection and the dermis of the vent region. These two extensions of the cloacal gland are separated from the main body of the gland by the paired muscles of the ventral body wall (musculus-pubo-ischio-tibialis) and are embedded in the connective tissue between the dermis and those muscles. These short, ventral extensions of the cloacal gland will be referred to henceforth as the ventral cloacal gland extensions.

More caudally these ventral cloacal gland extensions are joined by the downward growth of the main medial cloacal gland (figures 7 and 8). In transverse section the cloaca is now a horizontally flattened tube, and the cloacal glands immediately ventral to the cloacal wall are in intimate association with the supporting musculature of the latter, but have not yet penetrated the stroma of the cloacal wall.

In this same area, which is about 2 - 3 mm. anterior to the cloacal orifice, another type of gland, the abdominal gland, makes its appearance adjacent to the lateral edges of the ventral cloacal gland extensions (figure 8). The cloacal glands have increased in mass, and the tubules lateral to and below the cloaca now occupy, in transverse section, an area 10 mm. wide and 6 mm. deep between the cloaca and the ventral body wall.

The cloacal cavity is now beginning the transition from a horizontally flattened tube to that in which the main axis is in a dorso-ventral plane and in which slender ridges on either side of the large, central multi-ridged cloacal papilla project from the lateral surfaces of the cloacal wall into the cloacal orifice (figures 9 - 11). At the same time the only aspect of the anteriorly flattened cloacal cavity is that of two dorso-lateral horizontal folds of the cloaca.

The mass of the cloacal gland is largest in this region and gradually decreases in mass posteriorly. This decrease occurs first in the region adjacent to the vent opening, where the

abdominal glands gradually encroach medially into the region previously occupied by the cloacal glands (figure 14).

As stated above, most of the cloacal glands empty into the cloacal cavity through the ridges on the lateral walls of the cloaca, and some of them penetrate the smooth surfaces of the cloacal chamber. The cloacal glands terminate caudally a short distance posterior to the vent as two paired strands of glands lying next to the dermis in the midventral plane, separated by a thick cord of loose connective tissue (figure 19).

Histology of the Cloacal Glands

The tubules of this large gland may vary considerably in cross-sectional size, since they usually become progressively smaller as they approach their excretory ducts. This does not always hold, however, since some tubules are more or less uniform in size throughout until they suddenly decrease in size shortly before they terminate. Tubule diameter often exceeds 0.5 mm. and may be less than 100 μ in cross section, particularly where tubules penetrate the musculature of the cloacal wall. The tubules form a compact mass (figure 31), encapsulated in a fine sheet of connective tissue and held together by a fine connective tissue network; the tubules are moderately vascularized.

A typical tubule, with a cross-sectional diameter of 430 μ, has a unicellular internal lining of low cuboidal cells 15 μ in depth, resting on a thin basement membrane. The free surface of

these cells is usually distinct. The cytoplasm is composed largely of a coarse reticulum filled with droplets of a light green-staining mucoid substance, and the same material also fills the lumen of the tubule.

In active cells the nucleus is spheroid and may extend the entire depth of the cell. In some parts of a tubule the nucleus may be undergoing degeneration, being shriveled and lying at the base of the cell, and in other cells the nucleus may be absent. In those cells so enucleated, the cytoplasm, in addition, appears to be undergoing a dissolution, particularly at the free border which often presents a ragged profile. No free nuclei were observed within the lumen of these tubules and nothing was observed to indicate the ultimate disposition of the nuclei. Possibly they are phagocytized by phagocytic cells of the reticulo-endothelial system. The light green-staining material filling the lumen is not entirely homogeneous in substance; scattered through it are minute droplets of a darker green-staining substance. The tubules of these cloacal glands terminate on the tips of the ridges of the cloacal wall in low conical elevations (figure 32).

Anatomy of the Pelvic Glands

The glands which are collectively called here the pelvic glands present the greatest interpretive difficulty because they represent tubules which appear to produce at least three kinds of secretory products. The pelvic glands constitute the dorsal

portion of the cloacal glandular complex. Just above the roof of
the cloaca, the pelvic glands occupy an area which in frontal
section has the shape of a "Y" in which the arms are relatively
long, broad and curve outward slightly, and in which the stem is
divided, broad and short. Between the ventral portion of the
divided stem lies the dorsal longitudinal furrows of the cloacal
roof. The spaces lateral to the arms and stem are occupied by
the dorsal-most lateral portions of the cloacal glands.

The pelvic glands may be further differentiated into three
regions on the basis of the histological nature of the tubules.
Those glands occupying the arms and stem of the Y-shaped gland may
be called the anterior pelvic glands. The tubules of the stem
portion of the anterior pelvic glands extend caudally into the
portion of the pelvic glands at the caudal end of the cloacal
roof; these caudal-most tubules of the pelvic gland constitute the
posterior pelvic glands.

In transverse sections the anterior pelvic glands first appear
as large tubules on the dorsal side of the anterior tips of the
cloacal glands (figures 4 - 6). Connective tissue septa separating
these glands from the cloacal glands are limited only to those
sections drawn in figures 4, 5, and 6. Posterior to figure 6,
these septa gradually disappear. The anterior pelvic glands
extend as far caudally as the region depicted in figure 13. The
posterior pelvic glands constitute the remainder of the pelvic
glands shown in figures 14, 15, and 16.

The tubules of the anterior and posterior pelvic glands are not always clearly distinguishable from the tubules of the cloacal glands because the limits and interspersion of the tubules among these glands are not definite. These two groups of pelvic glands can be distinguished, however, from the cloacal glands by the nature of their histological and secretory characteristics. These pelvic gland tubules are of approximately the same diameter as the adjacent cloacal gland tubules and are about as straight. As the tubules of the anterior pelvic gland approach the cloaca, they become interspersed with the tubules of the cloacal gland.

Between the arms of the anterior pelvic glands is the relatively small mass of the median pelvic gland tubules shown by angled lines dorsal to the cloaca in figures 4, 5, and 6. Because they are separated anteriorly from the rest of the pelvic glands by connective tissue septa, the median pelvic glands are easiest to distinguish. More caudally the tissue septa are lost, but the median pelvic gland tubules can be distinguished, nevertheless, from the anterior pelvic and cloacal glands with which they become interspersed.

Histology of the Pelvic Glands

Anterior Pelvic Glands. The anterior pelvic gland tubules consist of a very fine outer connective tissue membrane overlying flattened fibroblasts, closely investing a thin, homogeneous basement membrane, and a lining of single, low to flattened

cuboidal cells whose intercellular boundaries are difficult to distinguish (figure 33). The content of the large lumen is the most distinguishing feature of these tubules, and is composed of at least two different secretions. One of these, the background substance, is a homogeneous, very light pale pink-staining or nearly colorless substance, and the other material is composed of irregularly-shaped granules varying in size from barely visible (under oil immersion) to over 15 μ in diameter. These granules stain uniformly bright orange.

The nuclei of the lining epithelial cells are ovoid to flattened; the particular shape depending upon the size and apparent activity or past activity of the cell. In those cells containing an ovoid nucleus, which may be as much as 23 μ across, the nucleus as well as the cytoplasm appears to be active, and in this condition the cell may have a depth of somewhat over 30 μ. The nuclei in these cells contain large dark granules. In those lining cells containing flattened nuclei, the nuclei have a shriveled membrane and the contained dark granules are more compressed, the whole presenting a picture of very dark shriveled nuclei lying against the basement membrane. The depth of these cells may be one-half or less of those previously mentioned.

The cytoplasm of the cells in different phases appears to be similar, except that in the larger cells it stains pale grey-pink, while in the smaller cells the cytoplasm stains considerably darker pink. Under oil immersion two definite regions within the

cytoplasm are seen to contain an orange-stained substance that corresponds very closely in staining qualities with the irregularly-shaped, bright orange granules seen in the lumen of the tubules. One of these regions is immediately adjacent to the basement membrane. In fact, the orange-staining substance, which is a very thin strand, sometimes appears to occur between the basement membrane and the outer connective-tissue membrane, but this may be an artificial condition created by the histological technique. The other region of the cell containing a thin strand of this orange-staining material is just below the free surface of the cell.

The cells containing shriveled nuclei, mentioned above, appear to contain greater amounts of this substance than the larger cells. Occasionally small, horizontally elongated blobs of this material may be seen within the central part of the cell cytoplasm. The light staining ground substance filling the lumen appears to be formed from the dissolution of the free cell surface.

The tubules of the anterior pelvic gland (figure 34) gradually decrease in size as they approach their terminal openings in the epithelium of the cloacal roof. As these tubules decrease in overall diameter, the cytoplasm of the lining epithelial cells disappears quite suddenly, just a short distance from where the tubules penetrate the cloacal wall mucosa. The investing tissue of the tubule in this region, where the secretory cells are absent, is heavily invested with very dark and flattened nuclei. Each of

these tubules terminates in a simple opening on the edges of the small, thin ridges which project from the cloacal roof into the cloacal cavity.

Posterior Pelvic Glands. The tubules of the posterior pelvic glands empty into the cloaca through the lower half of the walls of the dorsal furrow. In so doing, the tubules necessarily go around the lateral longitudinal fold of the cloacal wall (figure 35). The posterior glands empty into the cloaca through simple low papillae. The tubules of the posterior pelvic glands are not distinctly separated from those of the anterior pelvic glands, but they are distinguishable by their glandular epithelium and, more readily, by the nature of their secretory products. In other respects they are similar.

The difference in glandular products, although this may not be a constant difference, is that, in contrast to the variable, irregular-sized, orange-staining granules within the lumen of the anterior pelvic glands, the orange-staining granules within the lumen of the posterior pelvic tubules are far more abundant as minute droplets. There are relatively few of the large granules which are characteristic of the anterior pelvic tubules.

The glandular epithelium is essentially similar in appearance to that of the anterior pelvic glands. The posterior glandular epithelial cells are only slightly larger and their cellular limits are, in most cases, definable. The most striking difference between the anterior and posterior tubule cells lies in the manner

in which the posterior cells apparently form and secrete the orange-staining granules. In these posterior tubules there appear, at frequent but irregular intervals between the intercellular membranes of the cells, very dark, spindle-shaped basal structures which appear to be much shriveled nuclei. Closely surrounding the basal portion and sides of these nuclei is an investment of very dark orange-staining material which continues toward the free surface of the cell. In some instances the whole cell is involved, and in others only a thin dark strand leads away from the nucleus toward the free surface. That these dark bodies are, indeed, nuclei, can be shown by all the observable degrees of transition of the nuclei; from one which may be in the initial stage of shrinking to that of one in the stage described above.

The distal portion of these tubules shows the same reduction in size and other characteristics which were noted in those of the anterior pelvic tubules. One other difference between the anterior and posterior tubules concerns the difference in color in the homogeneous ground material within the lumen. In the posterior pelvic tubules this material is darker and has a greenish cast rather than a light pink one.

Median Pelvic Glands. The tubules of the median pelvic gland, which occupy the crotch of the Y, first enter the roof of the cloacal wall immediately caudal to the region of the urogenital papillae; they extend ventro-caudally through the moderately dense connective tissue of the dorsal cloacal wall and empty into the

cloaca through small conical elevations, which represent the cephalic ends of the cloacal dorsal ridges (figure 36). They also empty via the cloacal papilla through similar conical elevations. The tubules of this gland are only slightly smaller in diameter than the cloacal gland tubules and are very much convoluted, but they are distinguished easily by the lining epithelium which, because of the tubules' convolutions, appears often to nearly occlude the tubular lumen.

The cells of the median pelvic tubules, which are cuboidal to low columnar and range in depth from 20 μ to 38 μ, rest upon a thin basement membrane which is covered by an equally thin connective tissue membrane. The clear, homogeneous cytoplasm contains a very coarse reticulum. In a transverse section many of the nuclei can be seen to be resting on the basement membrane in one far corner of their respective cells, and in contact with the intercellular membrane. While it is not a completely constant characteristic, this displacement of the nucleus is one of the most distinguishing features of the median pelvic tubules. The nucleus is ovoid or pyramidal in shape, 10 - 15 μ in diameter, and contains very coarse granules.

The lumen is filled with a clear homogeneous material which takes a deeper green stain than do the cloacal glands. As the tubules approach the mucosa underlying the cloacal epithelium, the lining epithelium of the tubules undergoes a marked reduction in diameter. Concomitant with this change the cytoplasm and

nuclei of the lining cells undergo a similar reduction in size so that within the distance covered by three to four cells, the overall size of these cells may be reduced from 30 μ to about 12 μ with very little cytoplasm remaining. The walls of these tubules now appear to be composed entirely of closely packed nuclei.

Anatomy of the Abdominal Glands

In referring to the glands described below, the name, abdominal glands, unfortunately is misleading because in Dicamptodon these glands do not occur in the abdominal region. They receive their name from the fact that in some salamandrids they extend over the roof of the cloaca into the abdominal cavity (8, p.283). It is believed that these glands, like the hedonic glands of other species, play no part in spermatophore formation, but serve rather to stimulate the female during courtship. However, the histological characteristics of the abdominal glands in Dicamptodon differ markedly from the hedonic glands in plethodontids and salamandrids as depicted by Noble (8, pp.109, 118, 136). Perhaps a better name for these glands in Dicamptodon would be "cloacal lip glands." In lieu of a more complete investigation into the precise nature of these glands, I shall follow custom and continue to use the name abdominal glands.

The abdominal glands first appear anteriorly as one or two very large, easily distinguished tubules (solid black in drawings)

below the ventral dermis and adjacent to the ventro-lateral aspect of the cloacal glands (figure 8). Their anterior-most reaches are slightly posterior to the area where the ventral and medial portions of the cloacal glands meet. As seen from the figures, the abdominal gland mass increases in size more posteriorly, being interspersed with cloacal gland tubules (vertical lines in the figures). The abdominal gland tubules reach their greatest mass immediately adjacent to the lips of the cloaca in the region of the ridges of the cloacal aperture (figures 13 and 14). Posterior to these ridges, the mass decreases in size until it disappears a short distance beyond the caudal end of the cloacal lips. The abdominal glands extend nearly as far posteriorly as the cloacal glands.

The abdominal gland tubules, which are greatly convoluted, occupy nearly all of the subdermis region of the tumescent area surrounding the vent. In all probability they are mainly responsible for the somewhat swollen appearance of the vent region. They are concentrated most heavily just beneath the dermis, although they may extend dorso-laterally of the vent region into the cloacal glandular mass.

Histology of the Abdominal Glands

Histologically, the abdominal glands are quite striking in their large size and in the nature of their secretions which completely fill the cavities of their tubules, at least prior to the breeding season. These greatly convoluted tubules may exceed 1 mm.

in diameter, but are usually somewhat smaller. These large
tubules have blind endings and possess short ducts leading to
their terminal openings (figure 37). The abdominal gland tubules
are enmeshed in a stroma of loose connective tissue, blood and
lymph vessels. Each tubule is invested in a moderately thin sheet
of connective tissue which overlies a thin inner band of spindle-
shaped smooth muscle cells 3μ or less in width. Beneath this
muscle band is an extremely fine basement membrane upon which the
cells of the tubule lie.

The tubule cells are cuboidal or columnar in shape and have
their nuclei resting on the basement membrane. There is con-
siderable variation in the size of these cells in different
regions of the tubules. The smaller cells are located in or near
the blind ends of the tubules, whereas the tallest of the cells
occur within the major portion of the tubule. In some instances,
one side of a tubule is lined by tall columnar cells and its
opposite side possesses low cuboidal cells. The tallest of the
columnar cells may exceed 150μ in length, with a width of
$2 - 5 \mu$, and the smallest cells of the same tubules may be less
than 30μ in length. The tall columnar cells constitute a large
majority of the cells and appear to be the cells actively secreting
their products into the lumen.

The products of secretion take up the light red and green
stains of Masson's trichrome stain, and varying amounts of the
red-staining material appear within the cytoplasm as a very fine

reticulum, as small granules or as large irregular, anastomosing blotches. The anastomosing blotches contained in these cells usually extend down to and surround their respective basal nuclei. The rest of the cytoplasm is filled with a light green-staining homogeneous material in the form of minute droplets among the meshes of the cytoplasmic reticulum.

The intercellular membranes are distinct, by virtue of the concentration of fine red-staining droplets on their inner surfaces, but the free, dissoluting surface of actively secreting cells may merge with the lumen material so as to be indistinguishable from the latter. In some regions, where the cytoplasmic and lumen material contain relatively little of the red-staining secretions, the free surface of the cells is distinct.

The low columnar or cuboidal cells of these tubules seem to be in a stage of development and mild secretory activity rather than in a state of exhaustion or dissolution. These cells are characterized by containing relatively little of the red-staining cytoplasmic material. The nuclei in many of these cells stand out sharply because a large part of the surrounding cytoplasm is stained light green. The nuclei in all the secretory cells appear similar, being flattened-ovoid to nearly spheroid in shape. A few of them may be elongated and oriented in the long direction of their respective cells. These elongated nuclei may slightly exceed 20 μ in length, whereas the other nuclei range between 6 - 14 μ in diameter. Occasionally, a nucleus may be observed lying near the free surface

of the cells in much the same manner described previously (page 20) as occurring in the lining cells of the Wolffian ducts and in other tubule cells.

The contents of the lumen of the abdominal glands are clear and homogeneous in texture and vary in staining qualities as stated above. Apparently little meaning can be attached to the chemical nature of the lumen contents on the basis of their staining characteristics, since in identically treated adjacent serial sections on the same slide, the lumen of one section may be green, while the lumen material in the section immediately adjacent to it may stain red.

The ducts of these tubules, as stated above, are short and arise abruptly from the secretory portion of that part of the tubule which terminates just below the stroma of the cloaca lips (figure 37). These ducts penetrate the stroma and the flat, cuticle-free surface of the epidermis, which in the region of the cloacal lips is no deeper than 400 μ (figure 17). The transition is sharp from secretory epithelium to that of the duct, whose wall is a simple two-layered non-secretory epithelium. In some instances there is a slight constriction at the origin of the duct and the glandular portion of the tubule; between this constriction and the place where the duct narrows near its terminal opening, it may exhibit a slight bulb-shaped enlargement.

The opening of the duct is simple, lacking in any elevations.

The unmodified epithelium of the duct wall resembles that of the epidermis which it penetrates, but its layered nature, in contrast to that of the epidermis, is quite marked. The basement nuclei of the ducts are flattened and do not greatly exceed 50 μ in diameter, whereas the nuclei of the superficial cells are elongated perpendicularly to the duct wall and may easily exceed 150 μ in length. The free surface of the lining cells is ragged, but there is no indication that these cells contribute to the glandular secretions.

THE CLOACAL WALL AND ITS EPITHELIAL STRUCTURE

A study of the cloaca of several _Dicamptodon_ shows that the walls of the cloacal chamber are elastic and present an internal complex configuration of ridges. However, the internal ridges appear to be rather constant in the individuals examined. In the following description, the anterior portion of the cloaca is taken up first, followed by regions which correspond closely to those depicted in figures 4 - 19. The individual specimen being described is a neotenic male in which sperm are present in the Wolffian ducts as well as within the cavity of the cloaca; along with the sperm in the cloacal cavity is some cellular debris of unknown source, probably of intestinal origin.

The urogenital ducts (figure 24) and bladder open into the cloacal chamber just posterior to its anterior origin. In the region where these ducts empty (figure 4), the cloaca in cross section has the appearance of a much-indented triangle with the roof more or less flattened, and the steep-angled sides indented by irregular longitudinal furrows which form moderately thick ridges. A small, short, median ridge projects into the cavity from the cloacal roof. The Wolffian ducts enter at the lateral corners of the roof and the bladder empties at the ventral apex.

The epithelial lining of the cloacal cavity is nowhere simple. In the region of the urogenital papillae, the epithelium is two-layered at times, and appears to be pseudostratified in other

regions. Where the epithelium consists of two layers, it has a base of ovoid cells and a superficial layer of cuboidal to columnar cells. These latter cells nearly always contain a mucoid substance in the superficial half of the cell. Many of these mucoid cells are in the process of emptying their contents into the cloacal cavity.

Scattered irregularly through the superficial layer of epithelial cells are other cells, occurring singly or in small groups with darker cytoplasm than that of the mucoid-containing cells and with ciliated free borders. Moreover, only a part of the free border of one of these cells may be ciliated. Scattered patches of ciliated epithelium are particularly conspicuous through-out the region of the urogenital papillae (figure 38). However, the regions immediately adjacent to the openings of the many gland ducts are free of cilia.

In general, the sides and deep crypts of the ridges throughout the cloaca are the most densely ciliated; the cilia in these areas being slightly longer and more closely set together than elsewhere. The free borders of ciliated cells are very dense, and the cilia can be seen to have their origins in the cytoplasm a slight distance below the free surface of their respective cells.

Slightly caudal to the point of entry of the urogenital ducts, the shape of the cloacal cavity changes rapidly, so that in cross section it presents a flattened triangle whose apex is now directed dorsally. The apex of the triangular-shaped cavity is formed by a

low domed concavity (figure 7). The median pelvic glands empty into the cloaca through this region of the cloacal roof. Laterally there are several longitudinal ridges whose sides extend into two longitudinal cavities. The floor of the cavity is nearly flat, and only slightly indented by several small median furrows. Very few cilia occur either in the roof epithelium or in the lateral furrows, but the floor is completely ciliated, as are the edges of the ridges.

More caudally (figure 9), the ventro-lateral ridges become more pronounced. Most of these ridges are the internal cephalic portions of the external cloacal ridges. In this same area the dorso-cephalic end of the cloacal papilla projects deeply into the cloacal cavity as a large, median, arrowhead-shaped structure whose sides are also indented by the furrows of small ridges. The indentations of the roof of the cloaca are now nearly parallel with those of the floor configurations, and the lateral furrows have increased in size and remain a constant feature as far back as the caudal extension of the cloacal papilla (figures 9 - 13). Only the ventral surface of these lateral folds are ciliated, the roof of each fold and the one curved side being free of cilia (figure 39).

The sides and edges (excepting duct openings) of all the ridges are now heavily ciliated; however, the cilia on the edges are smaller (figure 32). The epithelial lining in this internal caudal portion of the cloaca is represented primarily by tall columnar cells. The epithelial lining here is similar to that of the more

anterior portion of the cloaca, except for the epithelium of the lateral folds where, as mentioned above, cilia are lacking. For the most part, this unciliated epithelium is reduced to a more compact layer of cells by a reduction in size of nuclei, as well as by a reduction in the depth in the cytoplasm of superficial cells. Relatively few cloacal glands empty into the cloacal chamber through these lateral furrows. The cloacal papilla and adjacent ventral ridges are traversed heavily with cloacal gland tubules.

As the external orifice of the cloaca is approached (figure 11), the cloacal cavity assumes a definite dorso-ventral elongation, and the cloacal papilla extends more deeply into the cavity. The cloacal lumen is not reduced in size, however, by the cloacal papilla, because the depth of the cavity in the same region increases to a much greater degree. This increase in depth is due primarily to a deep, median, narrow furrow which forms in the roof of the cavity (figure 12). The sides of this deep dorsal furrow are unciliated. The sides of the internal ridges, as well as those of the cloacal papilla ridges, are still densely ciliated. The outer surfaces of the ridges of both the walls and the papilla, however, are now ciliated only in small patches. The lower sides and ventral surface of the cloacal papilla are totally unciliated, as are the two ridges nearest the cloacal aperture. Thus, as the external orifice is approached (with one exception noted further on), the cilia of the cloacal wall are localized and reduced in size.

The anterior pelvic glands empty into the cloaca primarily through the epithelial tissue of this furrow. The furrow space in this particular Dicamptodon is completely filled with the secretory products of the aforementioned glands.

A few sperm are present in the cloacal cavity just forward of the exterior opening (figure 12); these sperm are adjacent to the tip of the cloacal papilla. The lateral furrows, which formed a prominent aspect of the cavity more anteriorly, are now reduced in size and possess characteristic epithelium and cilia. The lateral ridges of the wall and the cloacal papilla are becoming more thoroughly traversed by the cloacal gland tubules. Strands of smooth muscle arising from the cloacal wall penetrate through the papilla to its dorsal tip.

The cloaca has its greatest depth in the cephalic-most region of the external aperture (figure 13). In this region the apex of the dorsal furrow develops two small lateral furrows, which persist posteriorly for nearly the entire length of the orifice. About 14 ridges appear on both sides of the wall. A slight variation has been noted in the number of ridges among the several Dicamptodon examined. The cloacal glands empty into the cloaca throughout the remaining posterior extensions of these ridges.

Slightly anterior to the lips of the cloaca, the cell layers near the ventral aspect of the cloacal cavity gradually increase in number until, in the region of the orifice, the epithelium at the margin of the cloacal slit is stratified and resembles that of

the body covering elsewhere except for lacking a cuticle. The epithelium of the cloacal lips is considerably thicker, however, than the adjacent epithelium of the ventral body surface. The cloacal lip epithelium exhibits a variation in depth from about 100 μ to 210 μ (figure 37). The epithelium of the adjacent body wall exhibits less variation in thickness, varying from 40 μ to 70 μ in thickness. It may be noted in figures 17 and 37 that the abdominal glands empty their products into the curved lips of the cloacal aperture. Their terminal openings are restricted to the small area on the cloacal lips between the ventral-most limitations of the ridges of the orifice and the inner curved surface of the lips. This distance in the one specimen measured 1.5 mm. The cuticular surface of the body epithelium, which approaches the inner curved surface of the cloacal lips, appears to mark the outer limits of the abdominal gland openings. None of the glands typical of the body epithelium are present in the lips of the cloaca. The surface of the cloacal lips and the distal portions of the three ventral-most ridges in this region are composed of unmodified low columnar cells.

Except for a slightly darker staining reaction and containing more spheroid nuclei, the lining cells of the deep dorsal furrow and its lateral folds appear similar to those cells of the cloacal lips. The remaining surfaces covered by cilia are the bases of the ridges which are confined to the lower ventral portion of the walls of the cloacal aperture (figure 15). The cloacal glands now

open into the smooth dorsal walls of the aperture as well as
through the ridges.

Posteriorly, beyond the ridges, the abdominal glands and their
terminal apertures become reduced in number, and the region of the
lips is now largely penetrated by the cloacal gland ducts. In
contrast to the low conical elevations which mark the openings of
the cloacal gland tubules in the ridges and elsewhere, no such
papillae distinguish the abdominal gland openings on the lips of
the cloacal aperture. Instead, there is only a simple opening,
bordered by unmodified epithelium.

The lateral grooves in the dorsal apex of the roof become
reduced and disappear as the caudal end of the cloacal aperture
is approached. This feature is variable, however, since in other
neotenic Dicamptodon these lateral grooves terminate in a deep
dome-shaped cavity at the caudal end of the sperture. The melano-
phores which are present in the cloaca of the male are seen to lie
within the connective tissue immediately below the stratum germina-
tivum. In the region immediately posterior to the cloacal aperture
proper, none of the cloacal or abdominal glands empty onto the
surface, although tubules of both of these types extend beyond
this region.

ECOLOGY

One of the necessary parts of this study has been the collecting of Dicamptodon ensatus in their natural habitats. The field work of collecting these salamanders and maintaining them in captivity has resulted in some information, additional to that appearing in the literature, about the habits and life history of this species. Because little is known about this animal, I am presenting some of that information along with new information which I have acquired from field and laboratory studies.

Dicamptodon ensatus is one of five species of the family Ambystomidae which occur in Western North America; it is the largest terrestrial salamander in North America, adults sometimes exceeding 12 inches in length. It ranges in humid, forested, coastal regions from southwestern British Columbia through Washington, Oregon and California, down to Santa Cruz County; it has also been recorded from Idaho and Montana (10, p.49).

Like some other species of ambystomids, Dicamptodon is neotenic in parts of its range. In the mountainous area west of Corvallis, Oregon, it appears to occur more commonly as a neotene than as the metamorphosed form, but both types occur within the same region. Both forms have been taken from Mary's Peak at an elevation of 3,250 feet, and both occur at lower elevations. Dicamptodon, therefore, appears to be polymorphic. Moreover, this polymorphism seems to exhibit a clinal distribution, since in California, the

neotenic form is relatively uncommon and terrestrial individuals are fairly abundant. In Oregon, particularly in areas which have been thoroughly investigated, neotenes are generally far more common than are terrestrial individuals. In addition to this latitudinal clinal distribution, there appears to be an elevational one as well, since most Dicamptodon collected from higher elevations are terrestrial, whereas the majority of those taken at lower elevations are neotenic.

The evidence for the clinal nature of neoteny is based on general observations, sight records and by inspection of the collections of Dicamptodon ensatus in the Museum of Vertebrate Zoology, University of California, Berkeley, and in the Oregon State College Museum of Natural History, Corvallis.

Neotenic adults, depending upon favorable temperatures and humidity, have been taken in different localities at all times of the year except November and December. They may be active during these two months also, but high, swiftly-moving water has so far made it impossible to collect them during November and December. Because of the nature of their habitat, and possibly because they may become inactive at some times of the year, neotenic forms have not been taken throughout the year in the same locality. Like most other salamanders, Dicamptodon is usually not found in its habitat when the ground temperature is below 8°C. or the humidity is very low. Like normal Dicamptodon larvae, neotenic individuals normally live in cold-water mountain streams.

Between the early part of January and April 1, 1955, the
Oregon State Fish Commission maintained six fish traps in a small
stream in the foothills of McDonald forest, five miles north of
Corvallis. On February 20, 24, and March 1, 1955, three Dicampto-
don were taken from upstream traps. Between February 20 and
March 1, the mean average daily temperature of the water varied
between 7 - 8°C. It is interesting to note that all of these
animals were trapped while they were moving upstream. The signifi-
cance of these data is not clear, but it does indicate that
Dicamptodon may not be sedentary in its habits. This information
also leads one to speculate that Dicamptodon may be seasonal in
its movements. Two of the males trapped in this stream were killed
within one or two days after being trapped and both of them
exhibited active spermatogenesis, and their Wolffian ducts were
distended with stored sperm. Many neotenic Dicamptodon have been
taken by baited line from Rock Creek, Benton County (figure 40).

In only one instance known to me have neotenic Dicamptodon
been found in other than fast-moving streams, this instance being
their occurrence in a very small pond of about 100 feet in diameter
and 2 feet in depth (figure 41). This pond, 5 miles north of
Corvallis, and 1 mile west of Highway 99W, Benton County, is
located in low hills below McDonald Forest. It is fed by a small,
short tributary of a nearby stream which runs throughout the year
and which is the upper part of the stream in which the previously-
mentioned fish traps were placed. Between April 21 to 25, 1954,

I collected five full-sized neotenes from this pond by means of funnel traps. Several of these five individuals were near the maximal size of neotenic _Dicamptodon_ represented in the Oregon State College Museum of Natural History collection.

What factors may be involved in neoteny of _Dicamptodon_ remain largely unknown. The occurrence of terrestrial forms at high elevations is evidence, in part, that cold in itself is not the blocking agent of metamorphosis, as it is in the neotenous larvae of _Ambystoma_ _tigrinium_ in the Rocky Mountains (8, p.295).

Kezer[2] stated that metamorphosis was induced and completed by treating neotenic _Dicamptodon_ with thyroxine. I have kept neotenes at room temperature for one year without any metamorphosing effect, and several neotenes were maintained at 14°C. for a 4-month period without any metamorphic changes occurring.

Stebbins (10, p.54) speculates on the possibility that neoteny is common in areas where streams are high and steep-sided, implying that neotenous forms remain so because they cannot get out of the water and consequently would drown were they to metamorphose. I find this idea unacceptable for the regions investigated in this study, since all streams from which neotenous individuals were taken are not deep and offer every opportunity for an animal to climb onto land.

---

[2]Personal communication. Letter from James Kezer, Department of Zoology, University of Missouri (presently of Department of Biology, University of Oregon), dated February 6, 1954.

An interesting feature of these animals is that most of the
terrestrial individuals collected in this region are small (5 - 7
inches), whereas the neotenous individuals are almost uniformly
large. Lynn[3] suggested that a question of growth versus differenti-
ation may be involved in this species. From the foregoing, it
seems evident that failure to metamorphose is due to more than
one unknown cause.

Many difficulties were encountered in trying to maintain
neotenous forms alive in the laboratory. Neotenous Dicamptodon
were found to be extremely sensitive to chlorinated city tap water,
as well as to the metallic ions, most likely zinc ions, which may
be derived from the laboratory plumbing. Aquatic Dicamptodon
usually die if left in unfiltered tap water for three or more days.
The most rapid and noticeable effect of tap water is upon the
gills. In one instance, a mature neotenic individual escaped
from its container of filtered water into the running tap-water
bath where it remained overnight. In the morning, when it was
found and removed from the tap water, the gill filaments had
already become necrotic distally, and within two days all gill
filaments had dropped off, leaving only the stumps of the external
gill bars. New gill filaments eventually grew back. Aerating
the water and filtering it through a mixture of activated charcoal

---

[3]Personal communication. Letter from W. Gardner Lynn,
Department of Biology, The Catholic University of America, dated
July 5, 1954.

and oyster shells eliminated the problem of chlorine and metallic
ions.

SUMMARY

A study has been made of the reproductive system of the male neotenic salamander Dicamptodon ensatus in an effort to understand the main histological and anatomical features of the reproductive system of this animal.

Aquatic Dicamptodon were collected, killed, and their tissues and organs removed and prepared for histological study. Tissues were mounted as serially-spaced sections and stained with Masson's trichrome stain. An anatomical description of each organ or tissue has been given, followed by a histological treatment of those structures.

The cloacal regions of the male and female Dicamptodon are similar, but distinguishing features are present which permit easy identification of sex by using only external characters. The male is characterized by two external features: a series of prominent, parallel fine ridges in the anterior part of the cloacal orifice; and a cloacal papilla. This latter structure projects caudad from the internal cloacal cavity into the external orifice and has ridges similar to those of the cloacal wall. The female has a series of ridges also, but they are more widely spaced, not as parallel, and not as prominent as those of the male. A cloacal papilla is lacking in the female. Internally the cloaca of the male can be distinguished from the female by its urogenital papillae which are smaller than the oviducal papillae of the

female. In both sexes, urinary fluid has to flow into the cloacal cavity before it enters the bladder.

The gross anatomy and histology of the testes, sperm conveying ducts, epididymis, junctional tubules, and anterior and posterior portions of the Wolffian ducts are described. By comparing testes undergoing spermatogenesis with inactive ones, it was tentatively concluded that _Dicamptodon_ has a breeding cycle similar to that of _Taricha_ _torosa_. Each of the paired testes of _Dicamptodon_ is a single anatomical unit and does not exhibit the "multiple testes" aspect of some salamanders. However, the whole testis does not participate in spermatogenesis at any one time. Active and inactive portions of the testis constitute active and inactive lobules. The nature and development of the Sertoli elements appear to follow closely the sequence which occurs in _Taricha_.

The gross anatomy of the male reveals three kinds of glandular tubules closely investing the region of the cloaca: the cloacal, pelvic, and abdominal glands. The cloacal glands constitute the greatest mass and extend nearly the entire length of the lateral and ventral sides of the internal and external cloaca. The cloacal glands empty into the cloaca primarily through ducts opening onto the edges of the lateral ridges of the cloacal wall and the ridges of the cloacal papilla.

The pelvic glands lie over the roof of the cloaca and are differentiated into anterior, posterior, and median pelvic glands. Grossly, the pelvic glands have the shape of a "Y", the arms and

upper part of the stem constituting the anterior portion, and the base of the stem (cloacal cavity within the stem) being the posterior pelvic glands. The median pelvic glands lie between the arms of the "Y". The tubules of the pelvic glands open through short ducts into the roof of the cloacal cavity.

The abdominal glands are restricted primarily to the region underlying the epithelium of the lips of the cloacal aperture and open onto the smooth lip surface posterior to the ridged portion. The abdominal glands are considerably larger in tubule size than any of the other cloacal gland complex tubules. On the basis of the regions of the cloaca into which the various glands empty, it appears that only the cloacal and pelvic glands participate in spermatophore formation. The abdominal gland apparently plays little part in contributing to the spermatophore. Its function is unknown, but it is presumed to attract the female with its secretions.

The gross anatomical structure and histology of the cloacal wall is described. The internal configuration of the wall is made complex by many ridges and furrows which are present. The lining epithelium exhibits a variation in thickness and in the nature of the superficial and underlying cells. Cilia are present in different regions and vary in length and distribution from one region to another. They presumably aid in directing the movements of the sperm into the formation of the spermatophore.

The ecology of <u>Dicamptodon</u> is briefly discussed, particularly

the polymorphic nature of this species, and the habitats of neotenic individuals. The nature of neoteny in this species remains largely unknown. Low temperature, however, does not appear to be a major blocking agent of metamorphosis.

Maintaining neotenic _Dicamptodon_ in the laboratory has presented some difficulties associated with chlorinated tap water which also contains metallic ions. The effect of tap water containing these substances is lethal, if the animals are kept in untreated tap water longer than a few days. This difficulty was overcome by filtering tap water through activated charcoal and oyster shells.

BIBLIOGRAPHY

1. deBeer, G. R. Embryos and ancestors. rev. ed. Oxford, Clarendon Press, 1951. 159p.

2. Chase, Samuel W. The mesonephros and urogenital ducts of Necturus maculosus Rafinesque. Journal of morphology 37:457-531. 1923.

3. Dawson, A. B. The cloaca and cloacal glands of the male Necturus. Journal of morphology 36:447-463. 1922.

4. Francis, E. T. B. The anatomy of the salamander. Oxford, Clarendon Press, 1934. 381p, 25 plates.

5. Gray, Peter. The microtomists' formulary and guide. New York, Blakiston, 1954. 794p.

6. Hyman, Libbie H. Comparative vertebrate anatomy. 2d ed. Chicago, University of Chicago Press, 1942. 544p.

7. Miller, Malcolm R. and Marilyn E. Robbins. The reproductive cycle in Taricha torosa (Triturus torosus). Journal of experimental zoology 125:415-446. 1954.

8. Noble, G. Kingsley. The biology of the amphibia. New York, McGraw-Hill, 1931. 577p.

9. Pantin, C. F. A. Notes on microscopical technique for zoologists. Cambridge, University Press, 1946. 75p.

10. Stebbins, R. C. The amphibians of western North America. Berkeley, University of California Press, 1951. 539p.

ILLUSTRATIONS
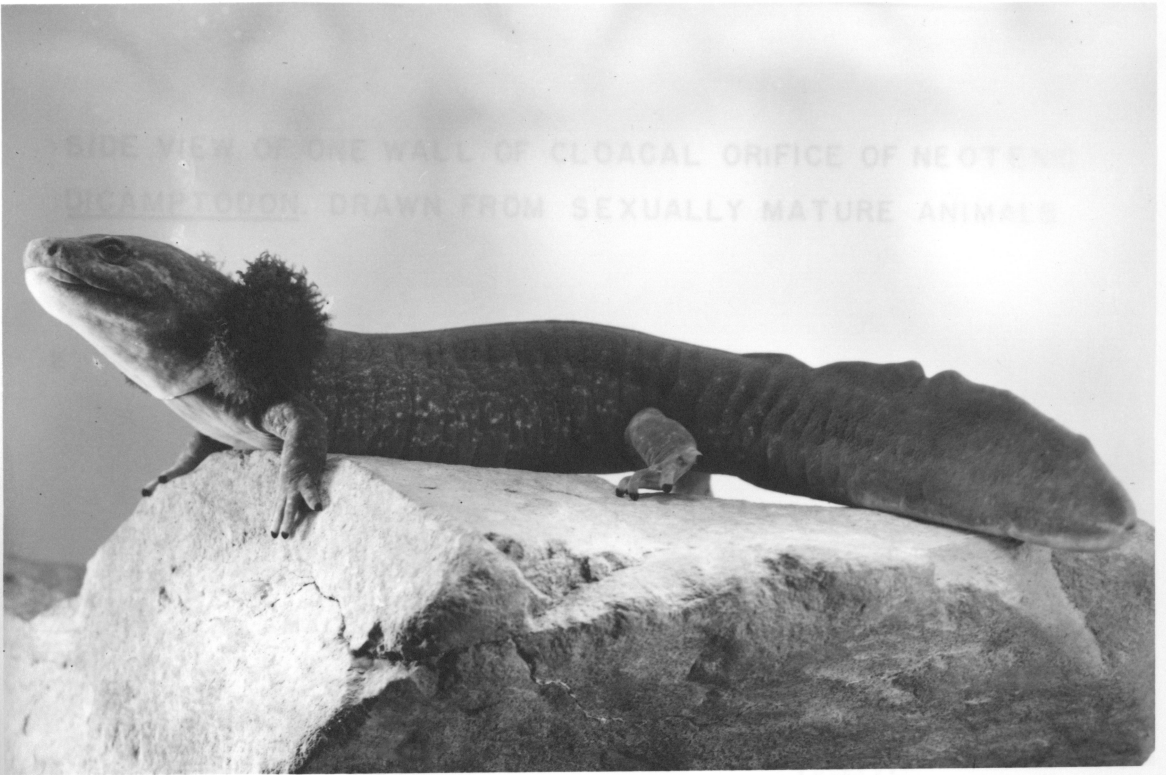
Figure 1. Neotenic _Dicamptodon_ _ensatus_ in aquatic habitat.
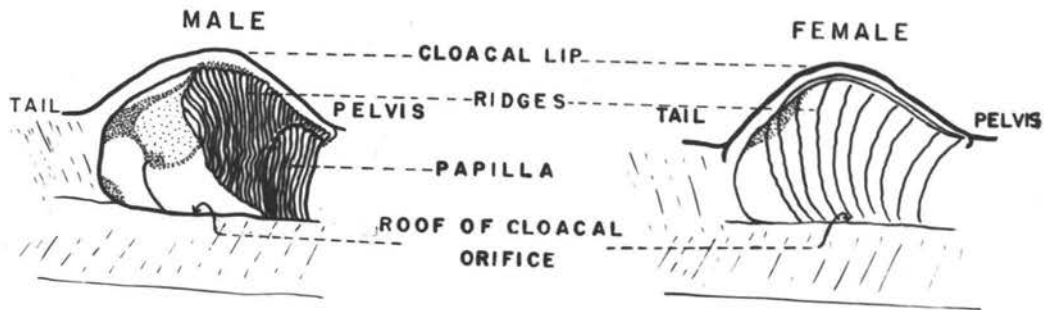
SIDE VIEW OF ONE WALL OF CLOACAL ORIFICE OF NEOTENIC
DICAMPTODON. DRAWN FROM SEXUALLY MATURE ANIMALS.

2
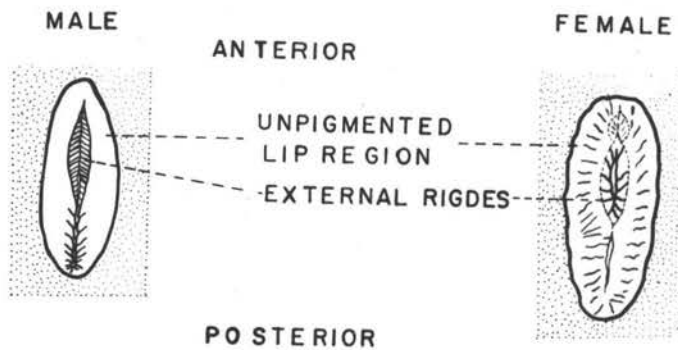
VENTRAL VIEW OF CLOACAL ORIFICE. DRAWN FROM
SAME INDIVIDUALS AS ABOVE.

3

Figures 4 - 19. Camera lucida drawings of spaced
serial transverse sections of
cloaca of male neotenic _Dicamptodon_.

4   5   6   7

8   9   10   11

12   13   14   15

16   17   18   19
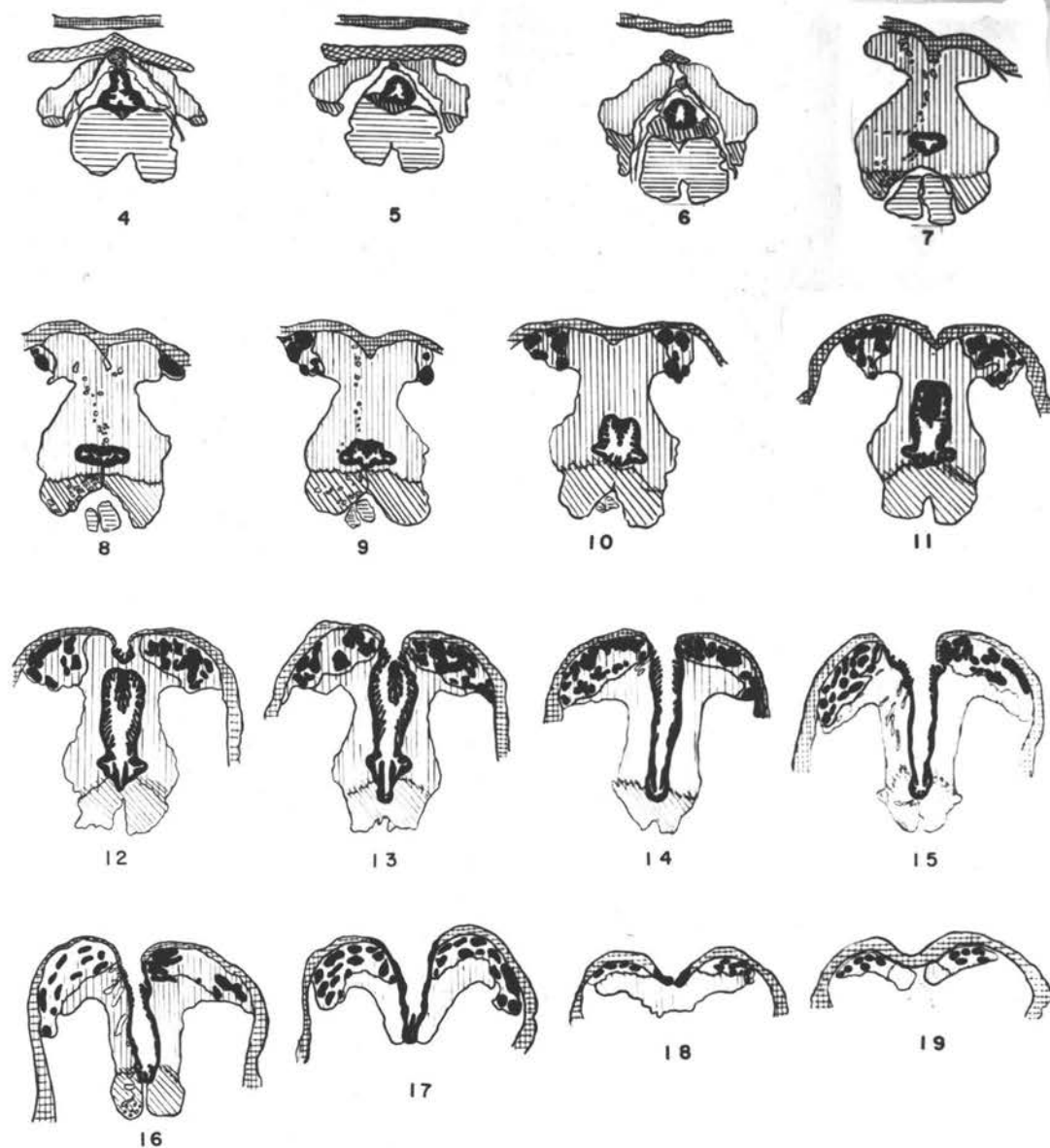
LEGEND

cloacal wall -----
cloacal papilla -----
skin -----
cartilage -----
bladder -----

abdominal gland -----
pelvic gland -----
cloacal gland -----
kidney -----

Figure 20. Transverse section of active testis.
        A - inactive lobule
        B - active lobule

Figure 21. Testicular ducts, mesial side.
        A - intralobular duct
        B - interlobular duct
        C - blood vessel
        D - beginning of efferent duct
        E - Sertoli cells

Figure 22. Epididymis.
        A - sperm within Wolffian duct
        B - ciliated renal ducts
        C - sperm within convoluted
           renal tubule

Figure 23. Frontal section of cloaca.
        A - urinary kidney
        B - intestine
        C - Wolffian ducts
        D - cloacal glands
        E - cloacal wall ridges
        F - dorsal furrow

Figure 24. Urogenital and Wolffian ducts.
        A - urogenital papilla
        B - urogenital duct
        C - urogenital tubules
        D - Wolffian duct

Figure 25. Sperm within convoluted tubule of
        urinary kidney.
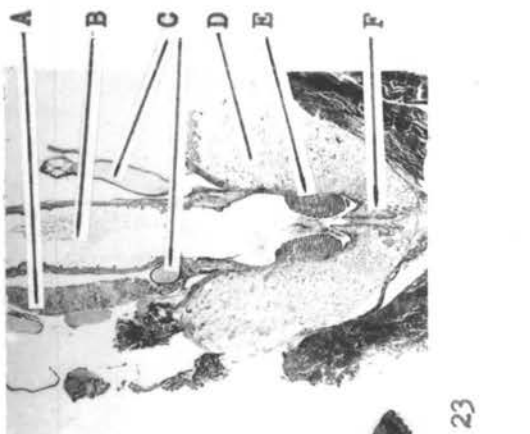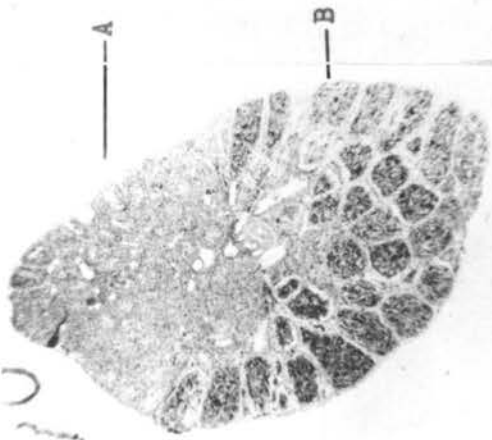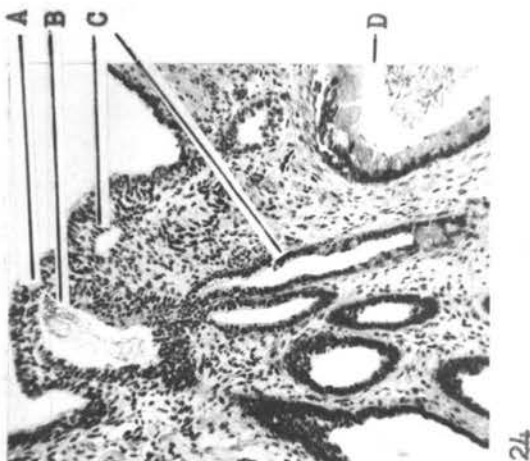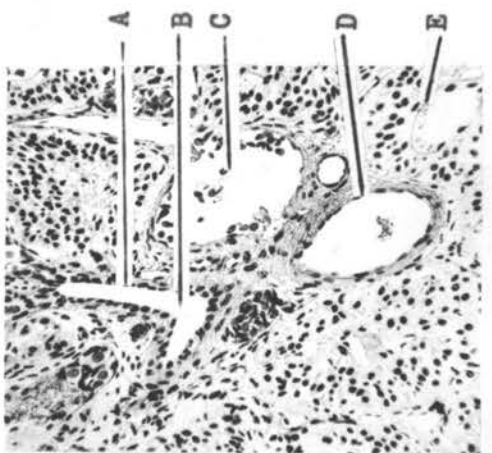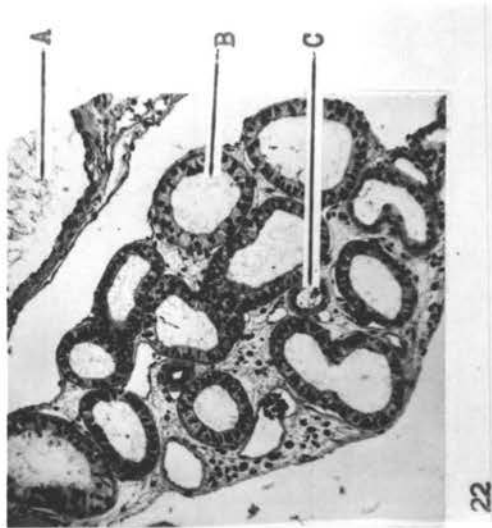
22

25

21

24

20

23
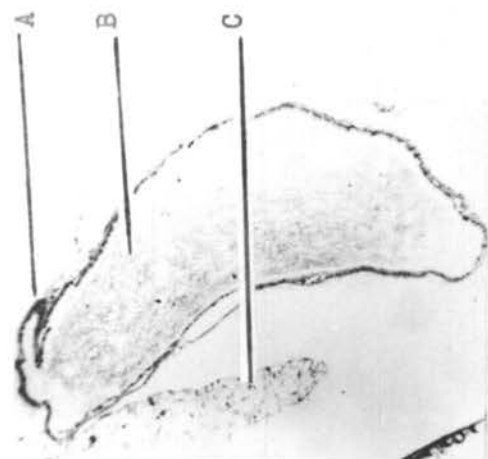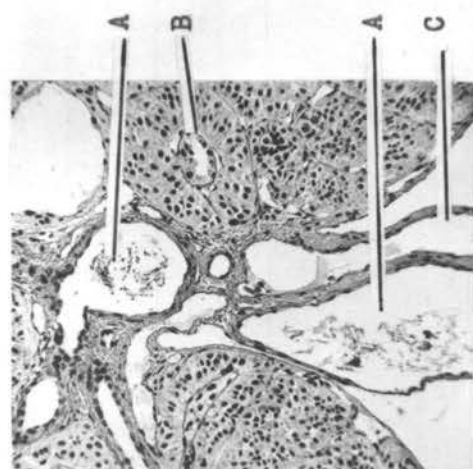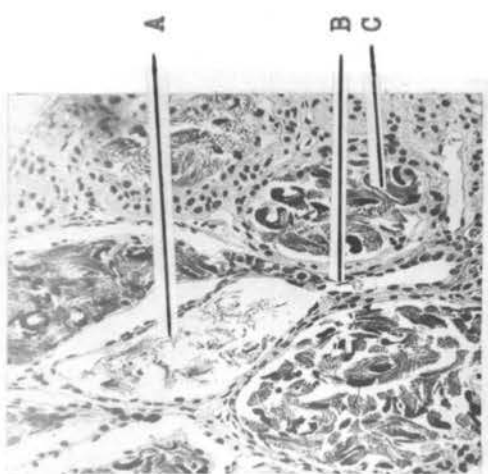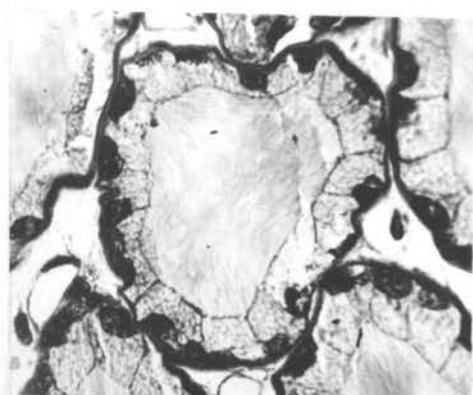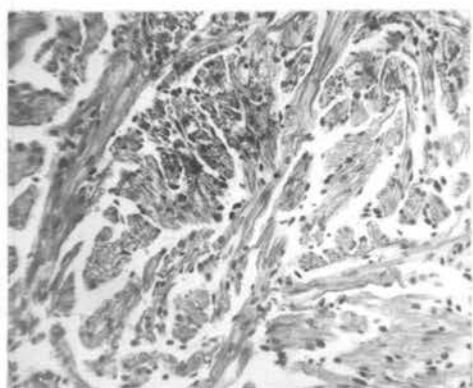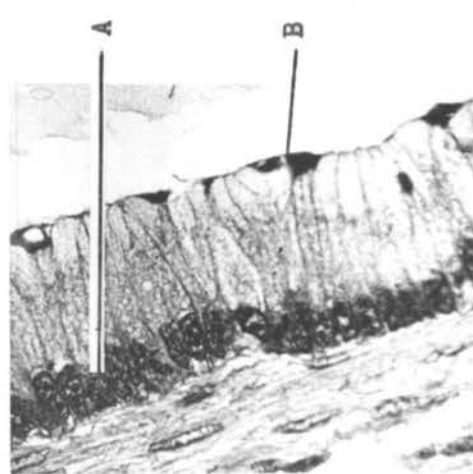
Figure 32.   Cloacal gland tubules terminating in low
                    conical elevations on cloacal wall
                    ridges.

Figure 33.   Anterior pelvic gland tubules.

Figure 34.   Anterior pelvic gland.
                    A - anterior pelvic gland tubules
                    B - cloacal gland tubules
                    C - urinary kidney
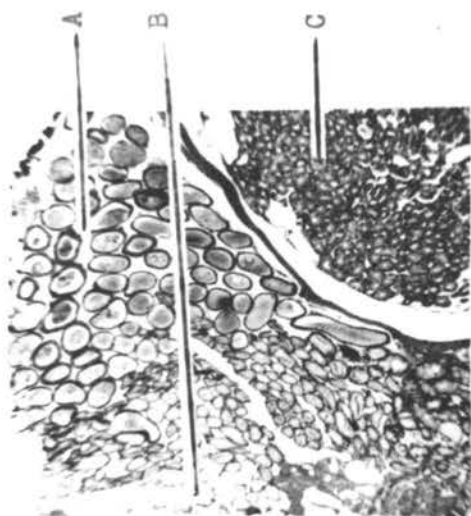
Figure 35.   Posterior pelvic gland tubules.
                    A - lateral longitudinal fold of
                          cloaca
                    B - posterior pelvic gland tubules

Figure 36.   Median pelvic gland tubules.
                    A - duct opening into roof of cloaca

Figure 37.   Abdominal gland tubules with ducts opening
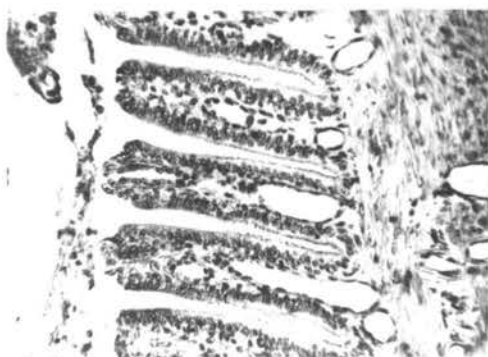   onto lip of cloacal aperture.
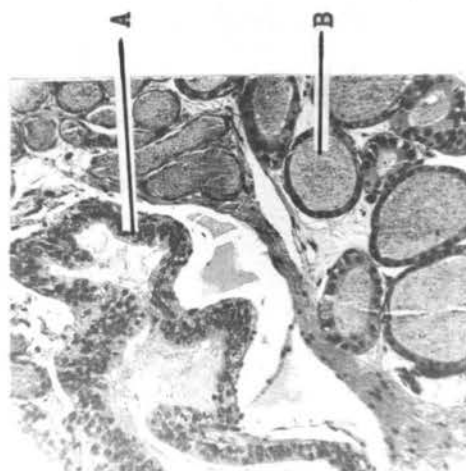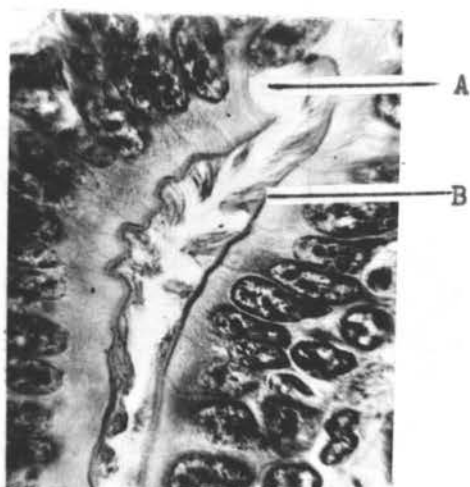
74
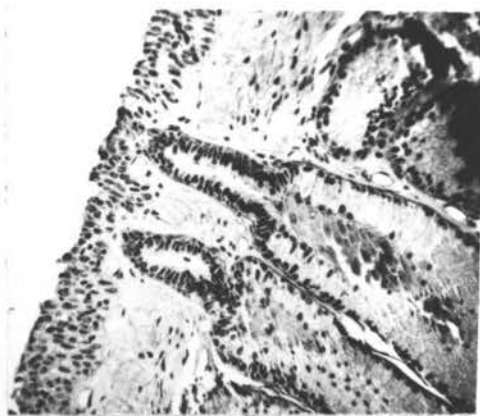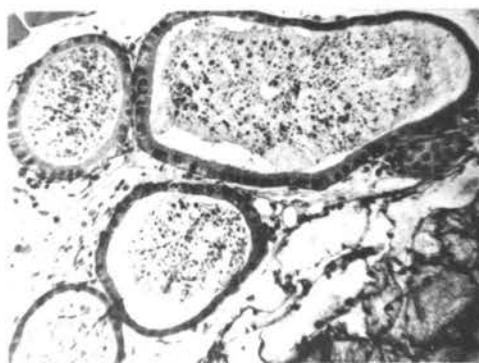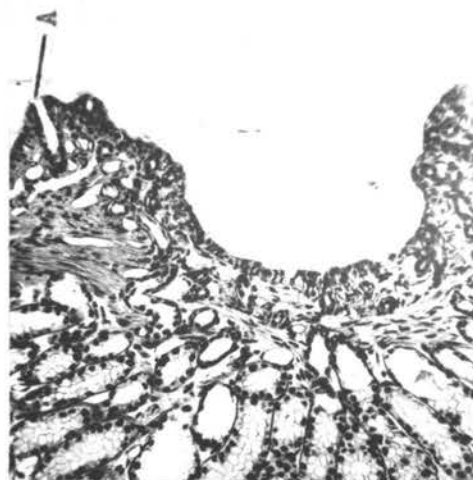
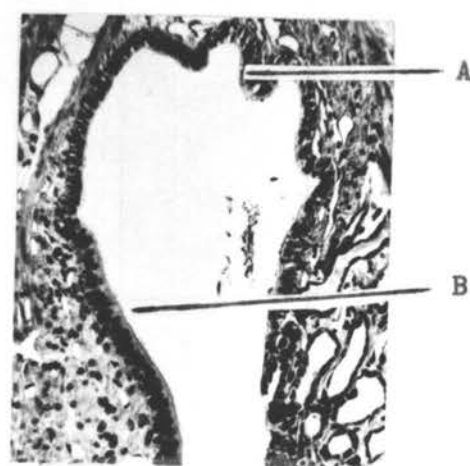Figure 38. Ciliated epithelium of
cloacal wall.
A - mucous cell
B - cilia

Figure 39. Lateral furrow of cloaca.
A - unciliated epithelium
B - ciliated epithelium

38

39

Figure 40. Rock Creek, Benton County. Habitat of neotenic
Dicamptodon.

Figure 41.   Pond 5 miles north of Corvallis in which neotenic
<u>Dicamptodon</u> live.