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Title AN INVESTIGATION INTO THE METAMORPHOSIS OF DICAMPTODON ENSATUS (ESCHSCHOLTZ)

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The investigation was conducted from January 1965 to March 1966. An intensive effort was made to obtain large numbers of animals, as it is believed that unavailability of specimens has been the limiting factor in the amount of research concerning this species. Two hundred and sixty-nine animals were collected. They are listed in the Appendix. It is desired that this list will serve as a source of reference for future investigators.

Four methods were used to obtain specimens. The one employed determined the size and form, larval or adult, of the animals taken. Effectiveness of the method used was largely dictated by weather and water conditions. The animals were maintained in water which had been filtered through activated charcoal and crushed oyster shells.

Although fair numbers of Dicamptodon were obtained, many died while in the laboratory. Larvae of all ages, when placed in filtered water, emptied the contents of their stomachs and fouled the water. This, coupled with cannibalism, resulted in an excessive loss of

specimens. Others died from unknown causes. Thus, the study was restricted by the small numbers of animals available for observation.

Nonbreeding larvae were injected with L-thyroxine and observed daily for evidence of transformation. In addition, second-year larvae were observed during normal metamorphosis. The sequence of events and their time relationships were studied. Only external changes, consisting largely of the atrophy of larval structures and pigmentation changes, were considered.

Some second-year larvae were maintained at 4° Centigrade, while others were kept in deep water, at room temperature, to determine the effects of low temperature and water depth on transformation. Neither blocked metamorphosis.

Data pertinent to size, age, and time of year at transformation were obtained. Second-year larvae, not subjected to thyroxine treatment and ranging from 111.50 to 166.25 millimeters in total length, exhibited metamorphic changes. Three were transforming when collected. One specimen metamorphosed three months earlier than the time formerly proposed for the species. The data of this study indicate that transformation begins in early spring and continues until the end of August. A series of first-year larvae, maintained throughout the investigation, showed no tendency to metamorphose.

Neotenes responded positively to intraperitoneal injections of L-thyroxine, their own thyroid homogenate, and pituitary homogenate

prepared from the glands of Taricha granulosa and Rana pipiens. One animal received pituitary homogenate prepared from its own gland and those of other neotenes. It exhibited no response over a seven-month period.

Metamorphic changes were induced in second-year larvae by treatment with neotenic thyroid homogenate.

The data, although tentative, indicate that tissue insensitivity is of minor importance in the metamorphic failure of this species. Pituitary derangement (lack of releasing factor) appears to be a more feasible explanation.

AN INVESTIGATION INTO THE METAMORPHOSIS OF DICAMPTODON ENSATUS (ESCHSCHOLTZ)

by

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AN INVESTIGATION INTO THE METAMORPHOSIS OF DICAMPTODON ENSATUS (ESCHSCHOLTZ)

INTRODUCTION

Very little is known about the metamorphosis of Dicamptodon ensatus. Information pertaining to this phenomenon in this species is based on the works of Kessel and Kessel (1943a, 1943b, 1944). The papers published in 1943 consist of a growth study of the first and second-year larvae respectively. They found no transforming individuals, but concluded that metamorphosis typically takes place in midsummer of the second year of life when the larvae are between 130 and 140 millimeters in total length. On 21 September they found an abnormally small, transformed individual. They stated that, " . . . it measured only 105 mm. in length and was doubtless a stunted specimen. The fact that it lacked a hind leg would substantiate this conclusion." All of their specimens were taken from a one-mile section of Corte Madera Creek, Mill Valley, Marin County, California.

Additional information on metamorphosis appeared in their 1944 publication. Four second-year larvae were confined in the laboratory for observation. Two of the larvae were kept in aquaria containing sand and shallow water. The other two specimens were held in aquaria containing only water, which was two and one-half and five inches deep, respectively.

The larvae retained in shallow water progressed through a "normal" metamorphosis which required 11 and 18 days for completion. The first sign of transformation in one of the animals was, ". . . a loss of prominence on the part (sic) of the caudal fin and also of the fringes of the gills." The other specimen showed initial caudal-fin atrophy two days subsequent to initial gill reduction.

The larvae exposed their snouts above the surface of the water and carried on buccopharynegeal respiration. The gills, however, were not exposed to the air until they were about one-fifth their normal length, and even then they were not allowed to become dry.

The marbled pattern of the adult did not become evident until the terminal stage of metamorphosis when the larval skin was shed. Kessel and Kessel (1944) stated that ". . . the skin was shed in several large sheets. This revealed the marbled markings of the adult salamander."

The two specimens which were denied shallow water did not complete their metamorphosis. They remained in a partial state of transformation for six months. When the water level was lowered, however, metamorphosis was completed within a week.

Kessel and Kessel concluded that ". . . the inability to get out of the water causes a physiological block to the completion of metamorphosis. It may be that those axolotl-like adults of this species which occur (sic) inhabit the larger streams where the water is

deeper or at least where the vertical banks hinder them from exposing themselves directly to the air."

Reed (1949) reports findings contrary to those of Kessel and Kessel concerning the size of second-year larvae at the time of metamorphosis. One transformed individual was taken near sea level on the bank of a small branch of Fogarty Creek, three-quarters of a mile south of the Lincoln Beach Post Office, Lincoln County, Oregon. It measured 96.5 millimeters in total length. As far as the writer knows, this is the smallest transformed individual on record. Reed also states that Dr. L. E. Griffin (Reed College, Portland, Oregon) informed him (personal communication) that he had collected many specimens in the middle portion of the Oregon coastal mountains and had never found an individual, either larva or adult, larger than approximately 110 millimeters in total length. The present study has revealed that larger specimens may also be found in this locality.

The above account is about the extent of the present knowledge concerning the size at metamorphosis, the age at metamorphosis, the time of the year of metamorphosis, or the sequence of events in the metamorphosis of the Pacific giant salamander. These phenomena will be dealt with in more detail in the discussion portion of this thesis.

At the onset it is very important that the reader distinguish

between the terms paedogenesis and neoteny, as these are often used synonymously to designate any urodele that is capable of breeding in the larval state. Earlier workers merely referred to animals exhibiting this phenomenon as axolotls. Since this has led to much confusion, the terms will be used as defined by Goin and Goin (1962):

(1) Paedogenesis is the retention of larval characters subsequent to sexual maturity, due only to genetic factors. It is a genetically fixed condition in which the tissues are insensitive to the secretions of the thyroid gland, normally bringing about metamorphosis in other forms. Trachystomes, proteids, and some plethodontids are examples.

(2) Neoteny is the retention of larval characters after sexual maturity because of environmental factors, such as diet, low temperature, or lack of iodine in the water. Many ambystomatids belong to this category. In contrast to paedogenic forms, these are still capable of metamorphosing if the environment is changed; such animals will respond to thyroxine treatment.

The present study has revealed that the large Dicamptodon larvae will respond to thyroxine treatment, and they will hereafter be referred to as neotenes.

The largest unmetamorphosed Dicamptodon on record appears to be the 286 millimeter specimen mentioned by Bishop (1943). It was collected at Oak Grove, Clackamas County, Oregon.

The smallest known neotene appears to be the 230 millimeter specimen (OSUMNH-1) taken at sea from the stomach of a fish (species unknown) in 1947. The animal was alive when discovered.

Neoteny is commonly manifest in urodeles which inhabit lakes of high mountain regions, while individuals of the species living at lower elevations exhibit a normal metamorphosis (Lynn and Wachowski, 1951; Noble, 1931; Snyder, 1956). Furthermore, it is well established that cold either inhibits the release of thyroxine or the ability of the tissues to respond in the normal manner. This, however, does not seem to be the case with Dicamptodon, for adults are commonly taken at high elevations (up to 5, 000 feet). Kessel and Kessel (1944) state that neotenes are probably more abundant in the central and northern portions of their range. They attribute this to an increase in rainfall rather than to a colder climate. Many earlier investigators held the belief that the steep sides of high mountain lakes forced the larvae to remain aquatic (Lynn and Wachowski, 1951). Kessel and Kessel (1944) subscribe to the same hypothesis, only in relation to streams having steep sides coupled with heavy rainfall. The writer questions the validity of such a hypothesis. During the present investigation, all neotenes were taken from streams that afforded every opportunity for the animals to emerge from the water if they were induced to do so. Moreover, this hypothesis does not account for the diminutive individuals found in the Oregon coastal mountains, an area of

excessive rainfall.

Lynn and Wachowski (1951) state that other environmental factors, such as oxygen tension and food supply, have been suggested as possible agents responsible for neoteny, but that little evidence has actually been advanced to support these hypotheses.

Schuierer (1958) states that the following factors are necessary for neoteny in Dicamptodon:

1. A year-round water supply.
2. Well oxygenated water.
3. A fairly constant water temperature.
4. An adequate food supply.
5. Protection afforded by the habitat.

Not until Gudernatch's (1914) discovery of the role of the thyroid in amphibian metamorphosis was there any progress made concerning the cause of neoteny. Subsequent to this discovery, numerous investigators have shown that the administration of thyroid substance, thyroxine, or various other iodine compounds to neotenic urodeles induces rapid metamorphosis (Ingram, 1928; Noble, 1931; Swingle, 1922, 1924). In light of this knowledge, experiments were undertaken to ascertain a basis for the failure of neotenes to transform in nature. Based on such experiments there are currently two major theories concerning metamorphic failure. These are endocrine imbalance and tissue insensitivity.

Endocrine Imbalance

The thyroids of neotenic Ambystoma tigrinum and Ambystoma mexicanum appear normal in that they contain abundant colloid, which although present is not released into the bloodstream (Blount, 1950; Swingle, 1922).

It is well known that the growth and development of the gland is largely influenced by thyroid stimulating hormone, which is produced by the pars anterior of the pituitary. Blount and Blount (1947) have adduced evidence that there are two distinct types of thyrotrophic activity in amphibians, one responsible for the storage of the colloid and the other responsible for its release into the bloodstream. Since the thyroid of certain neotenes contains abundant colloid, the storage factor must be produced in sufficient quantity. However, since the colloid is retained in the thyroid and not released into the bloodstream, the releasing factor must not be produced in sufficient quantity, not released from the pars anterior, or not produced at all. It has been shown that the thyroids of certain neotenic urodeles will release their colloid under the influence of normal pituitaries. Blount (1950) made reciprocal pituitary transplants between individuals of Ambystoma mexicanum (Mexican axolotl) and Ambystoma tigrinum (a readily metamorphosing form) when they were in the tail-bud stage. After they reached sufficient age, members of the former species

transformed under the influence of the pituitaries from the latter. Conversely, Ambystoma tigrinum carrying Ambystoma mexicanum pituitaries exhibited no metamorphic changes. Furthermore, Ambystoma mexicanum carrying homoplastic transplants showed no tendency to transform. He concluded that a pituitary derangement involving the releasing factor was responsible for neoteny in the latter species.

Tissue Insensitivity

As mentioned earlier, urodeles exhibiting complete tissue insensitivity are designated as paedogenic (Goin and Goin, 1962). These fail to respond to metamorphic substances regardless of concentration or dosage.

It has been shown that the thyroids of young neotenes will respond to the injections of anterior pituitary substance; in older specimens, however, the glands no longer seem capable of responding (Lynn and Wachowski, 1951).

Swingle (1924) found that if he removed the thyroid glands from three Ambystoma tigrinum neotenes and placed them into the pleuroperitoneal cavity of one of the animals, transformation, in a few cases, could be induced. The injection of the glands from two specimens was without effect. He concluded that metamorphic failure was due to an exceptional insensitivity of the tissues to the individual's

own thyroid action. These urodeles should not be called paedogenic, for even if they will not respond to the product of their own thyroid, metamorphosis can be induced if a sufficient dose from another source is administered.

The endocrine system of Necturus appears to be normal, metamorphic failure being due to extreme tissue insensitivity (Charipper, 1929; Charipper and Corey, 1930; Grant, 1930, 1931; Grant, Clapp, and Ruby, 1932; Swingle, 1922). The same seems to be the case with Proteus and the other perennibranchiates that have been studied (Lynn and Wachowski, 1951).

There is a definite need for scientific research concerning metamorphosis of the Pacific giant salamander. In fact, this species has been so largely neglected that any of its activities may be fruitfully investigated.

This investigation concerned itself with the following points:

1. The sequence of events and their time relationships during induced and normal metamorphosis of non-breeding larvae.
2. The effect of deep water on the transformation of second-year larvae.
3. The effect of low temperature on the metamorphosis of second-year larvae.
4. Tissue insensitivity and endocrine imbalance in relation to neoteny.
5. Minimum and maximum length of neotenes.

MATERIALS AND METHODS

Collecting

Adults of this species are rarely taken, but the larval form is common throughout western Oregon (Graf, Jewett, and Gordon, 1939). The small, nonbreeding larvae are more readily obtained than the large, sexually mature individuals. The method used in collecting determined the size of the specimens obtained.

Four methods were employed to acquire animals. They were arbitrarily named the line method, the hand method, the trapping method, and the road method.

The line method is mentioned by Stebbins (1954), and was introduced to the writer by Robert M. Storm, Oregon State University, who has collected many neotenes in the following manner: A large piece of fish or bloody meat is tied to a rock or other object which, when tossed into a large, sluggish, pool-like area in a cold, permanent stream, will carry the bait to the bottom. After a period of waiting (sometimes two and one-half hours), the animals may reveal themselves from their hiding places, such as from under large rocks, fallen logs, undercut banks, or other objects on the stream bottom. A small piece of meat, tied to a weighted string, is cast directly in front of the animal as it approaches the baited rock. The bait is generally taken with an exceptionally fast-forward motion and a

sideways slashing of the head. The animal can be pulled in slowly and secured in a dipnet, for it will usually retain a tight grip on the bait until after it emerges from the water.

This method is ineffectual when the water is high and exhibits cloudiness or turbidity, for one must see the animal. Attempts to capture neotenes during January, February, and March 1965, when the water showed these characteristics, yielded no returns. Storm has taken many specimens by the line method, during the summer and fall when the water was low and clear. On a single occasion he captured 14 neotenes from one large pool. Over the years this technique has been the most successful for obtaining large larvae.

Fifteen neotenes were taken by a slight modification of the line method. A small piece of fish was attached to a weighted monofilament line and placed directly below falls in Mill Creek, one mile north of Logsden, Lincoln County, Oregon. The water was low and clear.

The hand method, as the name implies, consists of overturning rocks and other objects situated in the stream. As the animals are sighted, they are guided into a dipnet.

The method was most effective for taking the smaller larvae, ranging up to approximately 170 millimeters in total length. When these smaller individuals were disturbed, they usually moved around very little and were easily manipulated into the net. This was not

the case with the larger ones, for they are agile swimmers, immediately seeking cover when disturbed. One specimen, however, 236 millimeters in total length, was taken by the hand method. It was captured with considerable difficulty, and required about 25 minutes before it was secured. Moreover, this method was effective for the rare adult form, but for these one should also look under objects along the stream bank and on the forest floor.

The hand method was most effective in the spring, summer, and fall when the water was low and clear. It did, however, yield a few animals during the winter months when the water was high and turbid. The greatest number of specimens were taken by the hand method.

The trapping method was the only way that neotenes could be obtained during the winter months. Ten funnel traps, constructed of 1/4 inch mesh wire, were utilized. Each trap was two feet long and eight inches in diameter. A funnel, having a three-inch opening, was fastened to each end. Unlike Ambystoma gracile, bait was required to obtain Dicamptodon.

Sheldon Johnson (Oregon State University) has taken as many as 70 individuals of the former species in an unbaited funnel trap. A variety of baits were used as follows: smelt, liver, crayfish, red snapper, and pork steak. Smelt was the most effective, with liver and crayfish yielding some returns. None was taken on red snapper,

but one was caught with pork steak. It has been the experience of the writer that Dicamptodon could not be taken with rancid bait. If the bait was not changed approximately once a week, it acquired a fungus growth, even in the cold streams where this species is found. Only two small larvae were taken in funnel traps. This method was not effective for these small individuals.

The traps were placed in small streams which flowed through second growth Douglas fir and were overshadowed by riparian vegetation, including alder, birch, and salmonberry.

Method of Retaining the Animals in the Laboratory

After the specimens were collected, they were transported to the laboratory in glass jars containing water from the source of capture. Various containers were used to hold the animals, including styrofoam ice chests, fishbowls, porcelain pans, plastic containers, and aquaria. All specimens were kept in water that had been filtered through activated charcoal and crushed oyster shells to remove harmful ions and add calcium. Aeration of the water was discontinued when transformation became evident. Gravel was provided so the animals could emerge.

Larvae of all ages, especially first and second-year larvae, emptied the contents of the stomach when placed in filtered water. This behavior continued for about one week. During this period.

the water was changed twice daily. If it was not, it acquired a pungent odor and resulted in an excessive loss of specimens. The smaller larvae were particularly sensitive to the fouled water and perished rapidly. Neotenes could tolerate this situation for a longer period of time, but they also died if this precaution was not taken. After the initial period, the water was changed twice weekly for the neotenes, once a week for the second-year larvae, and daily for the first-year larvae.

The animals ate well in captivity. First-year larvae were fed entrycheid worms, earthworms, and Daphnia. Neotenes and second-year larvae were fed primarily earthworms, but tadpoles, crayfish, and frog muscle were provided occasionally. They would also eat salamanders (except Taricha), including their own species.

Larvae of all age classes, even though well fed, exhibited cannibalistic tendencies. Ideally, they should be kept in separate containers. Larvae of about equal size were kept in the same container, but tails and appendages were often consumed. Neotenes of the same sex were held in separate containers. When two were placed together, each assumed a position at the end of the receptacle, and thus exhibited territorial-like behavior. If one approached the other, a battle ensued. A male and female were kept together without incidence. They laid near each other, rubbed each other, and even laid on top of each other, but made no attempt to fight. These large

animals appear quite lethargic, but they are capable of moving with incredible speed. They may readily remove an appendage or tail with their razor-sharp teeth and larvae were taken with an appendage or tail missing. Many possessed scars on their fleshy lips, dorsal surface, or sides. They should be handled with a dip net, for they will snap at a finger if given the chance.

Experiments Pertaining to Metamorphic Failure

The first step was to determine if the tissues of the large Dicamptodon larvae were insensitive to thyroxine, and to ascertain if this was a factor in the retention of larval characteristics throughout life.

Skin Grafts

Pigmentation of the integument was used as a criterion for determining tissue insensitivity. During transformation, the more or less dark coloration of the larva changes to the brilliant, gold and black mottled pattern of the adult.

Skin from large, nontransforming individuals was grafted onto second-year larvae. The latter were subjected to thyroxine treatment, the hypothesis being that if the tissue was not insensitive, the grafted integument would transform concomitantly with the host. Uhlenhuth (1917) performed homoplastic grafts between the skin and

eyes of Ambystoma punctatum larvae. After thyroxine treatment, the tissues transformed with the new host. He sutured with silk thread but gave no precise method.

Three methods of attaching the graft were attempted: glueing, suturing with tantalum wire, and suturing with silk thread.

Eastman 910 Adhesive, chemically known as methyl 2-cyano-acrylate, was employed in the nonsuture technique. Polymerization, which occurs with pressure, was aided by a slightly moist alkaline surface. It has been used previously on mammals with good results. Jesse, et al. (1964) made homoplastic skin grafts on both humans and dogs, while Healey, et al. (1964) closed bronchial stumps following pneumonectomy in mongrel dogs. The writer knows of no previous attempts to use the adhesive on urodeles.

Since neotenes were difficult to obtain, second-year Dicamptodon and Ambystoma gracile larvae were utilized to devise a suitable technique. Homoplastic grafts were made in the following manner:

The animals were anesthesized in a solution of MS 222 (1/3, 000 concentration).[1] A piece of skin, approximately 15 millimeters square, was removed from the top of the head of a larva (A). From another animal (B) a section of skin, slightly larger than the first piece, was taken from the same area of the head. The head of A was

[1] Throughout the investigation a 1/3, 000 concentration of MS 222 was used for anesthesization. Hereafter, the concentration will not be given.

then dried with absorbent paper, and the integument surrounding the recipient bed moistened with dilute potassium hydroxide. The adhesive was applied, in as small a quantity as possible, to the skin around the recipient bed. The larger section from B was then placed directly over the exposed area so that only the edges overlapped onto the intact integument of A. Small forceps were used to apply moderate pressure, for about 30 seconds, by squeezing the edges together. In this manner a strong bond was obtained, for the animal could be lifted by the graft without any separation occurring. The animal carrying the graft was placed in a culture dish immediately after the operation. The dish, the bottom of which contained absorbent paper moistened with distilled water, was transferred to a 16^{o} C. refrigerator for one week.

Thyroxine Treatment

Each second-year Dicamptodon larva carrying a graft from a large nontransforming individual was placed in a solution of L-thyroxine, 1/35,000 concentration.[2] Gravel was provided to enable the animal to emerge. The solution was changed daily.

Moreover, the thyroids were removed from neotenes, homogenized, and then injected intraperitoneally into the same individual

[2] Hereafter, L-thyroxine of 1/35,000 concentration will be referred to only as thyroxine.

from which they were taken, or into second-year larvae. Two neotenes were also thyroidectomized and injected daily with L-thyroxine.

Thyroidectomy was performed with the aid of a zoom binocular microscope (0. 7 by 10X): The animal was placed in a culture dish containing MS 222. Anesthesia required 30 to 45 minutes, depending on the size of the specimen. It was then placed, ventral surface upright, on a dissecting plan, into which the MS 222 was poured. This measure was taken to prevent the animal from awakening during the operation. Since the gular fold is unattached to the neck (Figs. 1, 18), it was lifted anteriorly and pinned to the wax in the bottom of the pan. The thyroid could be seen under the skin of the neck. It consists of two small masses, one on either side, near the insertion of the geniohyoid and rectus cervicis muscles (Fig. 3). Ventrally, they were partially covered by the branchiohyoid muscles. The integument which covered the glands was cut and laid back. The overlying fascia were then separated with a small pair of forceps. This exposed the gland, which was gripped with the forceps at its most posterior point and lifted gently. It was then separated from the underlying fascia with the point of a sharp scalpel. This freed the gland, except where it was attached at each end. One must be careful not to cut any of the close-lying vessels, as blood will cover the gland, making it difficult to remove the mass intact,

or even find the small organ in the large blood clot. Next, the posterior end of the thyroid was gripped with the forceps and the tissue was snipped at each end of the gland. It was then lifted from the animal. The above procedure was repeated for the remaining mass. Although histological sections were not made, there appears to be no isthmus of tissue connecting the bilobed thyroid. There is, however, an extensive vascular connection.

Immediately after the operation, the animal was placed in clean filtered water. The entire gland was then homogenized in eight milliliters of distilled water and kept in a small vial at 4^{o} C. No specimens were lost using the above technique.

Pituitary Homogenate Injections

Neotenes received daily injections of pituitary homogenate. They were observed daily for metamorphic changes. Thus, one could determine the response to thyroid stimulating hormone (TSH), which regulates the release of the colloid, thyroxine, from the thyroid gland (Allen, 1932; Blount, 1950; Charipper and Corey, 1930; Etkin, 1955; Grant, 1931; Ingram, 1929).

Pituitary homogenate was prepared as follows: adult Taricha granulosa and Rana pipiens were decapitated. Their pituitaries were removed and placed in distilled water in a one to one ratio, one pituitary per milliliter of water. After a number were taken, they

were homogenized and kept in vials at 4° C. Intraperitoneal injections were administered after the animals were anesthesized and hypophysectomized. Hypophysectomy was performed with trephines.

One neotene received pituitary homogenate, prepared from its own gland and glands of three neotenous larvae, and was observed for transformation signs. In this manner one could determine if neotenous pituitaries contained a sufficient amount of TSH to induce metamorphosis by releasing thyroxine from the thyroid.

Induced Metamorphosis of Nonbreeding Larvae

Second-year larvae were collected from small streams and brought into the laboratory. Each animal received daily, intraperitoneal injections of thyroxine. Observations were made daily to ascertain the sequence of events and their time relationships during the atrophy of larval structures. Pigmentation of the integument was studied with the aid of a zoom binocular microscope.

Normal Metamorphosis[3] of Second-Year Larvae

Second-year larvae, collected during June and July of 1965, were brought into the laboratory. Some were placed in containers which were provided with gravel and filtered water. The specimens were

[3] Normal metamorphosis implies that the animals were not subjected to thyroxine treatment.

observed daily with both the unaided eye and the microscope for metamorphic changes. Eleven animals were maintained at 4° C. to determine if this temperature would block metamorphosis.

Others were held in five inches of water at room temperature. Kessel and Kessel (1944) report that deep water prevents transformation in this species beyond partial gill reduction. Their two specimens that were denied shallow water remained at this stage for six months. When the water level was lowered, transformation was completed within a few days. As mentioned previously, they hypothesized that neotenic individuals inhabit the larger streams, and that deep water and vertical banks are major factors in their metamorphic failure.

RESULTS

Normal Metamorphosis at Room Temperature

Ten second-year larvae, ranging from 112.20 to 143.35 millimeters in total length, were observed during transformation. (Appendix A numbers 57, 98, 102, 106, 110, 112, 114, 115, 173, 133).[4] Since they exhibited a similar sequence of events, the animals will be considered collectively. The metamorphic process was divided arbitrarily into eight stages for descriptive purposes: (1) pigmentation changes, (2) formation of adult snout, (3) eye bulging, (4) formation of adult tail, (5) gill reduction, (6) gular-fold fusion, (7) formation of eye-stripe depression, (8) skin shedding.

1. Pigmentation changes. Pigmentation of the integument is a variable character. Many larvae are uniform in dorsal coloration, being almost black in appearance; others have yellow blotches present. Venter coloration ranges from almost white to dark gray. Yellow spots occur on the gular and pectoral regions.

Gold pigment, prominent in the adult, is also present in the larvae, although in less quantity and evident only under the microscope. Scattered haphazardly over the dorsal surface, in no particular pattern, it appears as small golden flecks (Fig. 4), often more

[4] Hereafter, all animals will be referred to by number. They are listed in Appendix A.

numerous on the head and near the base of the dorsal tail fin.

The majority of the specimens showed pigmentation changes three or four days prior to alteration of the snout. This consisted of an increase in the number of the flecks. By the time the gills had begun to atrophy, or shortly thereafter, the flecks assumed a definite pattern. They were arranged in small circles over much of the dorsal surface (Fig. 5). This change was first exhibited, in some animals, on the head and near the base of the dorsal tail fin. With respect to the gold pigment, certain areas of the dorsal surface did not show any change. These became the dark mottled regions of the adult.

The flecks, arranged in definite circles, gradually enlarged until they became confluent and appeared as complete rings of gold (Fig. 6). The animals looked brighter, and the areas which became the dark mottling were barely visible, for here the flecks were sparse and showed no definite pattern. They gradually widened, and by the time the gills were atrophied, gold pigment had completely filled the areas not destined to be the mottled regions (Fig. 7). A dark depression, located middorsal, from the region of the gills to the rear appendages, remained free of the gold pigment. It was, however, invaded by gold approximately six or seven days after the final molt, at which time, the animal appeared completely adult.

2. Formation of adult snout. The snout changes markedly during

transformation. Larvae possess a more or less shovel-shaped rostrum, and prominent lip folds are present on the upper and lower jaws (Figs. 1, 2, 12). The rostral shape is due largely to a flat, shelf-like area, lateral to each eye, which extends anteriorly to the external nares (Fig. 2). Hereafter, this area will be referred to as the "shelf".

To understand the formation of the adult snout, one must observe the atrophy of the above-mentioned structures. The first metamorphic change, evident to the unaided eye, occurred in the region of each external nare, and consisted of a reduction of the anterior-most portion of each shelf (Fig. 13). About five days later, the upper-lip folds began to abate. The shelves and upper-lip folds diminished from an anterior to posterior direction. They disappeared approximately ten days after shelf atrophy was initiated. The lower-lip folds began to atrophy shortly before, or at the same time of complete shelf and upper-lip fold reduction. They were absorbed along their entire length. Complete abatement occurred in about three days. The adult snout was formed approximately 13 days after initial reduction of the shelves.

3. Eye bulging. The degree of eye bulging in nontransforming larvae is variable. Some show no bulging, while others exhibit it slightly.

The eyes began to protrude two to three days following the

commencement of shelf atrophy, and they gradually became more prominent. In about ten days, they bulged approximately 75 percent that of the adult state (Figs. 14, 15, 16). The final protrusion occurred six to seven days after skin shedding was completed (Fig. 17).

4. Formation of adult tail. Dicamptodon larvae are of the typical stream type, possessing a dorsal tail fin which extends only to the sacral region (Fig. 8).

Approximately three days after stage two began, the tail fin reduced at its base (Fig. 9). It continued to diminish in a posterior direction (Fig. 10), resulting in the formation of the adult tail in about four days (Fig. 11).

5. Gill reduction. Initial gill atrophy was hard to detect. Each animal had to be scrutinized prior to transformation, because the length of the branchial filaments varied greatly from one larva to another. Specimens were also taken which had large, bushy filaments on one side but possessed filaments approximately one-third as long on the other. Moreover, some lacked filaments altogether on one stump while others had a club-shaped filament on each.

One explanation for these anomalous conditions could be that the larvae nip at each others' gills, and it has been observed in the laboratory. Knowing their cannabalistic habits, the writer would not be surprised to find this occurring in nature.

Initial gill reduction took place about six days after the shelves

began to atrophy. Four days later, the branchiae appeared as stumps, having very small filaments (Fig. 14). In five to six days, all that remained was a small, pigmented mass on each side of the neck (Figs. 15, 20). These disappeared in three to five days. Thus, gill atrophy required 13 to 16 days.

6. Gular-fold fusion. The larval gular fold is not attached to the underlying skin of the throat (Figs. 1, 18).

Initial fusion of this structure took place about seven days subsequent to the beginning of stage two. Attachment occurred near the midline (Fig. 19), proceeded laterally (Fig. 20), and became complete shortly before the pigmented gill masses were absorbed (Fig. 21).

7. Formation of eye-stripe depression. Larvae possess a yellow stripe, though sometimes faint, which extends from the eye to the gill on each side (Fig. 2). Approximately 11 days after the beginning of stage two, a slight depression was evident along the length of the stripe. This "eye-stripe depression" became prominent progressively and reached full development by the time the gills were reduced to pigmented masses. The yellow pigment was no longer evident.

8. Skin shedding. Skin shedding started about eight days after the shelves began to atrophy. This molt consisted of small fragments of skin which loosened from the body and appendages.

Six larvae shed larger pieces of integument (about one centimeter

in diameter) five to seven days after the first molt. The other four specimens did not undergo a second molt.

All animals exhibited the final molt approximately ten days subsequent to the first molt. The integument was shed in two to three large pieces.

Endocrinologists consider metamorphosis to be complete when the larval skin is sloughed in one or more large pieces (Noble, 1931). This, however, was not the case in the Dicamptodon, for two events took place six to seven days subsequent to the final molt. The eyes protruded more (see page 25) and the dorsal depression was invaded by gold pigment. Therefore, approximately 28 days elapsed from the time pigmentation changes were first noted until the adult form was attained (Figs. 17, 24).

During transformation, the larvae left the water for long periods of time, spending from 30 minutes to 12 hours out of the water. They were never seen out of the water until the shelves began to atrophy, at which time, or shortly thereafter, they emerged and assumed a rigid stance on the gravel. The body was held in an S-shape with the tail curved to one side. The only signs of life were the movements of the gular region, and slight up and down rhythmic motions of the head. Animals have been seen day after day on the gravel and in the same position. However, since they were not observed overnight, one cannot be sure that they did not reenter the water.

The animals showed transformation changes shortly after they were brought into the laboratory. Specimens 114 and 123 exhibited shelf reduction two days after they were collected. Seven larvae (98, 102, 106, 110, 112, 115, 133) showed pigmentation changes between July 13 and 20. Animal 57, taken on June 19, manifested pigmentation changes June 22 and shelf atrophy June 25.

Three larvae (124, 134, 144), showed no sign of metamorphosis. They were alive at the termination of the study.

Metamorphosis in Nature

Three larvae (59, 71, 228) were transforming when collected. Larva 59 was in the early stages of metamorphosis, for the gold flecks were arranged in circles (Fig. 5) and the shelves were in the initial phase of atrophy (Fig. 13). Transformation had progressed further in animal 71. The flecks were confluent, appearing as wide rings. The shelves were considerably more reduced and the tail fin was atrophied at the base. The gill filaments looked like they had diminished slightly, and the eyes bulged to a small extent. One could not be certain, however, about the eyes and gills, for the animal was not observed before transformation. The gular fold had fused about one-half way between the midline and the gill on each side. The specimen appeared as in Figure 22. Larva 228 exhibited the greatest degree of transformation. The gills were reduced to pigmented masses; the

gular fold was completely fused, except adjacent to each gill mass (Fig. 20); the shelves and tail fin were completely atrophied; and the eyes bulged considerably.

Metamorphosis at 4° Centigrade

Eleven larvae (104, 108, 126, 135, 136, 137, 138, 140, 141, 142, 143) were maintained at 4° C. to determine if transformation would be blocked. They were placed in the refrigerator July 8. All the specimens showed metamorphic changes within one week. Eight animals died before completing transformation. Three larvae (104, 108, 141) metamorphosed, but the process was extended considerably, requiring approximately 146 days. They passed through the same sequence of stages as did the larvae at room temperature.

Larvae Denied Shallow Water

Twelve larvae (145, 146, 147, 148, 149, 150, 152, 154, 156, 157, 158, 159) were kept in plastic receptacles containing five inches of water. No gravel was provided. Nine of the animals died before any indication of metamorphosis was noticeable. Larvae 113 and 116 had shown no sign of transformation and were alive when the study was terminated. Animal 145 did transform. Since this is the only Dicamptodon larva, known to the writer, to metamorphose when deprived of shallow water, its transformation will be considered in detail.

It was placed in the container of water on July 8. The first indication of metamorphosis consisted of a constriction in the area of each external nare, July 15 (Fig. 13).

Two changes were evident July 21: (1) the gold flecks had increased in number and were arranged in definite circles (Fig. 5), (2) The eyes bulged slightly more than in the larval condition.

The upper lip folds began to atrophy on July 25. Some of the gold flecks had enlarged, became confluent, and appeared as gold rings (Fig. 6). By July 27, the tail fin had reduced at the base (Fig. 9). The shelves were approximately one-half their original size, and the eye-stripe depressions were barely visible.

The shelves were reduced to about one-fourth the normal size by July 29. More of the gold flecks had become confluent. The animal was considerably brighter, and certain areas which were to become the black mottled regions of the adult were evident. No gill reduction or gular-fold fusion had occurred.

On August 3, the eyes protruded about one-half that of the adult state. The shelves were near complete atrophy. By August 5, they had disappeared, along with the upper lip folds. The lower lip folds had begun to abate, and the gular fold was attached at the midline.

The eye-stripe depressions were fully developed by August 6. Furthermore, the branchial filaments were reduced slightly.

On August 8, the eyes were bulging approximately three-fourths

that of the adult state. The lower lip folds were reduced to about one-half their original size. The rings of golden pigment had widened considerably, and the animal appeared very bright; dark mottled areas were prominent. The dorsal depression did not escape encroachment of the gold pigment and appeared as a dark, thin line, extending about two-thirds the way down the back. In addition, the dorsal tail fin was reduced one-half way between the base and the tip.

On August 9, the lower lip folds appeared as a small, thin line on each side of the lower jaw, and by August 11 they had atrophied completely. The golden rings were even wider and, except for a thin, dark line in the dorsal depression, the pigmentation resembled that of the adult. The gular fold was unattached only at a small area adjacent to each gill. The branchiae were approximately three-fourths their original length.

Small pieces of skin were shed on August 15. The animal made no attempt to surface. Instead, it rested quietly on the bottom of the container.

By August 18, the tail fin was completely atrophied (Fig. 11). The gills were about one-half the normal size.

Four large flakes of integument were sloughed from August 21 to 25.

By August 26, the gills were reduced to stumps, each having very small filaments.

On September 8, the skin was shed in one large piece. The gular fold had completely fused, and each gill appeared as a small pigmented mass. The degree of eye bulging showed no change since August 8.

On September 9, the animal made a continuous effort to leave the water, swimming to the surface, settling to the bottom, and then again swimming to the surface. At this time the water level was lowered and gravel provided. The specimen emerged, and assumed a rigid stance.

The eyes were protruding to the full extent by September 12. Thus, transformation was completed 67 days after initial shelf atrophy.

Reaction of Nonbreeding Larvae to L-Thyroxine Injections

Eight animals, ranging from 85. 5 mm to 186 mm in total length, were injected daily with thyroxine. Specimens 5, 13, 16, 27, 40, 42 and 43 received injections of one-half cc. each; the number of injections administered to each animal were 6, 15, 23, 16, 3, 3, and 3, respectively. Larva 32 received 15 injections of one-quarter cc. each.

All of the animals died before transformation was completed. Four, (5, 13, 16, 32) however, reached the stage where the gills appeared a s small, pigmented masses (Fig. 15).

The first indication of metamorphosis consisted of pigmentation changes which occurred three to six days after the first injection. The only exception was larva 31 which exhibited initial shelf reduction seven days subsequent to treatment. It showed pigmentation changes three days later.

A constriction in the area of each external nare became evident six to eight days following the first injection. The shelves were absorbed in three to five days. The upper lip folds atrophied simultaneously with the shelves, but lower lip fold reduction occurred two to three days after the shelves began to abate. Four to five days were required for their absorption.

Skin sloughing started seven to thirteen days following treatment, but three animals shed integument on the eighth day.

The eyes began to bulge nine to twelve days after the first injection. Three specimens exhibited this phenomenon on the ninth day.

Gill reduction occurred from the sixth to the fifteenth day, and tail-fin atrophy was initiated seven to fifteen days following treatment.

The dark mottling of the adult coloration was first noted from the 15th to the 18th day, while the gular fold became attached at the midline between the 14th and 16th day. It was completely fused four days later. By then, the gills were reduced to very small pigmented masses.

The reader can see from Appendix A that there was much variation in the total length of the specimens treated. Also, the number of injections were not held constant. This experiment was conducted so the writer would become familiar with the sequence of events and their time relationships during transformation, and the normal metamorphosis of the species could be more accurately analyzed.

Minimum and Maximum Length of Neotenes

1. Minimum length. Prior to the present study, the smallest larva known to the writer to contain mature ova was the 230 millimeter specimen mentioned previously (see page 5).

On March 9, 1965, two individuals were taken. One was an adult male measuring 260 millimeters in total length, while the other, a larval female, measured 208 millimeters in length. They were captured together in a submerged funnel trap which was situated in a small pool approximately four feet in diameter by two feet in depth. The pool was located in Woods Creek, five and one-half miles west of Highway 20, Benton County, Oregon. It was formed by a fallen moss-covered log about three feet in diameter which was partially damming the stream. Moreover, other logs, ranging from two to four feet in diameter, had fallen over the stream at this point. Decaying logs, very damp moss, and fern coverage, along with alder, Douglas fir, and salmonberry formed the surrounding habitat. Since

numerous springs and small tributaries flow into Woods Creek at this point, the area remains damp even during the dry part of the year.

The specimens were taken into the laboratory for observation. On March 14, the adult was found dead and was subsequently dissected to confirm sex and to locate the thyroid gland. It possessed an enlarged vent and testes in addition to greatly swollen archinephric ducts. Upon microscopic examination (430X), mature sperm were discovered in the archinephric ducts and at the cloacal aperature. The animal was definitely in the breeding condition.

The 208 millimeter larva died March 28, and its gonads were examined. Ova 5.6 to 6.0 millimeters in diameter literally filled the pleuroperitoneal cavity. Using this specimen as a criterion, any larva 208 millimeters or greater in total length was considered neotenic. It seems highly probable that the larva and the adult would have bred if they had not been disturbed.

2. Maximum length. Bishop (1943) reports a 286 millimeter neotene which was collected at Oak Grove, Clackamas County, Oregon. This appears to be the largest specimen on record. Robert M. Storm, Oregon State University, however, has collected 12 neotenes which exceed this length. All but one came from large pools in Rock Creek, Benton County, Oregon, near the vicinity where Highway 34 crosses the stream. One 312 millimeter specimen

(OSUMNH-1197) was taken here. Another individual (OSUMNH-1713) measured 310 millimeters in total length and was taken from a small stream located approximately one-half mile north of Lewisburg, Benton County, Oregon.

During the present study three neotenes (171, 264, 264) were obtained which exceed 286 millimeters in total length, the largest being 296 millimeters long.

Skin Grafts

Many problems were encountered in making the skin grafts.

The nonsuture technique was wholly unsatisfactory. Although a tight bond was initially acquired, it would separate completely in a period of time ranging from 30 minutes to 24 hours. The adhesive would first lift at its periphery and this would then proceed toward the point where the two pieces of skin came together. The monomer, being the viscosity of water, flowed onto the recipient bed when pressure was applied to the edges of the integument. This alone might prevent proper healing. The nonsuture technique was discontinued, as no successful grafts could be accomplished.

Tantalum wire also gave poor results. For some unknown reason, the grafts were never retained for longer than three days. The graft either separated completely or became unattached at two or more places.

Suturing with silk thread proved the most effective, but problems were also encountered. The major ones were infection and failure of the silk to hold the skin in place long enough for it to become attached.

Only two larvae were successfully grafted with integument from neotones. They were placed in separate containers and covered with thyroxine solution. Animal seven, 19 days after thyroxine treatment, regurgitated earthworms into the solution during the night. It was found motionless the following morning. A faint hearbeat was evident for 11 hours after the discovery, but death ensued. No change in the pigmentation had occurred. Ten days later animal eight was found dead in the solution. It had, however, changed considerably. The gills were reduced to small, pigmented masses and the integument had attained the characteristic mottling of the adult. The gold pigment appeared as small, wide rings in both the specimen's skin and that of the graft. Thus, it seemed that the integument of the neotene was not insensitive to thyroxine.

No other skin grafts were attempted, because injecting neotones with thyroxine solution proved a more effective technique.

Reaction of Neotenes to Thyroxine Injections

Animals three and 31 were thyroidectomized and then injected[5]

[5]Daily injections of 1/2 cc. each were given; hereafter the term "injection" will imply this dosage and frequency, unless otherwise stated.

with thyroxine solution. Thirty-two and 35 injections were administered respectively. The following metamorphic changes occurred. The number after each change designates the days subsequent to the initial injection when the change was first observed:[6]

1.	Shelf reduction	12
2.	Gill reduction	14
3.	Pigmentation changes	14
4.	Eye bulging	15
5.	Upper lip fold reduction	18
6.	Skin shedding	20
7.	Lower lip fold reduction	21
8.	Tail fin reduction	22
9.	Gular fold fusion	30
10.	Dark mottling	31

Both died in the terminal stages of transformation when the gills were reduced to pigmented masses, animal three, 32 days and specimen 31, 35 days after treatment. Number three showed severe hemorrhaging just prior to death. Blood was present at the mouth and cloacal aperature.

The gravel was utilized for long periods of time (see page 27). In fact, animal three was never seen in the water 14 days prior to death. While on the gravel, they assumed the characteristically rigid stance.

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[6] Hereafter, a number following the metamorphic change designates the days subsequent to treatment when the change was first observed. When more than one animal is considered, the average is given to the nearest day.

Reaction of Neotenes to Thyroid Homogenate Injections

Specimens 33, 39, 49, and 51 received injections of their own thyroid homogenate prepared from the entire gland.

1.	Pigmentation changes	6
2.	Shelf reduction	12
3.	Skin shedding	14
4.	Gill reduction	18
5.	Tail fin reduction	18
6.	Eye bulging	23
7.	Upper lip fold reduction	25
8.	Lower lip fold reduction	31

Transformation was not completed. Fusion of the gular fold did not occur, nor did the mottling become evident. These neotenes also used the gravel for long periods of time. Animal 49 was alive at the termination of the study and appeared as in Figure 23.

Reaction of Second-Year Larvae to Thyroid Homogenate Injections

Two specimens were injected with thyroid homogenate prepared from neotenous glands. They were not thyroidectomized prior to treatment.

Animal 12 received 13 injections (0.812 of the gland) of homogenate prepared from the thyroid of animal 31.

1.	Pigmentation changes	8
2.	Shelf reduction	9

Death occurred 13 days after treatment. The larva was not seen out of the water.

Specimen 62 received 12 injections (1/4 cc. each) of homogenate prepared from the gland of animal 51; 0.375 of the thyroid was administered.

1.	Pigmentation changes	5
2.	Shelf reduction	9
3.	Eye bulging	14
4.	Tail fin reduction	17
5.	Skin shedding	17
6.	Upper lip fold reduction	17
7.	Gular fold fusion	19
8.	Lower lip fold reduction	20
9.	Gill reduction	20
10.	Dark mottling	38

Fifty-seven days after treatment, the animal was found dead in the water. Transformation was near completion, as the gills were very small, pigmented masses. The gular fold was fused, except for a small area adjacent to each gill mass. The gold pigment appeared as wide rings and the mottling was evident.

Reaction of Neotenes to Pituitary Homogenate Injections

Nine animals were injected with pituitary homogenate prepared from the glands of Rana pipiens. One neotene received homogenate prepared from Taricha granulosa and Rana pipiens pituitaries. Another specimen was injected with homogenate prepared from the glands of neotenes, including its own pituitary. The 11 larvae treated were hyphosectomized prior to experimentation. Transformation changes are listed after each animal:

Animal 180 received one injection (1 cc.).

1.	Pigmentation changes	9
2.	Skin shedding	13
3.	Gill reduction	16
4.	Tail fin reduction	36

The animal was alive when the study was terminated. It appeared as in Figure 23 except no shelf atrophy or eye bulging had occurred.

Animal 181 was administered two injections (1 cc. each).

1.	Shelf reduction	11
2.	Skin shedding	11

Death occurred 11 days after the first injection.

Animal 179 received two injections (1 cc. each).

1.	Pigmentation changes	6
2.	Gill reduction	12
3.	Shelf reduction	15
4.	Tail fin reduction	15
5.	Eye bulging	20
6.	Skin shedding	43

The specimen died 47 days subsequent to treatment. Many of the gold flecks on the head and back were confluent, appearing as golden rings. The dark mottling, however, was not evident to the unaided eye. The gills were approximately one-half their original length, while the shelves were reduced about one-fourth. The tail fin had atrophied completely, and the eyes were bulging slightly more than in the larval condition.

Animal 175 received two injections (1 cc. each).

1.	Pigmentation changes	7
2.	Skin shedding	10
3.	Gill reduction	11
4.	Shelf reduction	13
5.	Eye bulging	27
6.	Upper lip fold reduction	35
7.	Tail fin reduction	39

Death ensued 41 days after the first injection. The gold flecks had increased in number and were arranged in circles. The gills and shelves had reduced to approximately one-half their original size. The upper lip folds were reduced anteriorly. The eyes were bulging about one-half as much as the adult state, and the tail fin was reduced at the base.

Animal 178 was administered three injections (1 cc. each).

1.	Pigmentation changes	10
2.	Shelf reduction	10
3.	Gill reduction	13
4.	Skin shedding	17
5.	Tail fin reduction	38

Death occurred 38 days after treatment. The gold flecks were arranged in circles, and the shelves were reduced about one-fourth. The gills were approximately one-half their original length. Tail fin reduction had just begun.

Specimen 177 received three injections (1 cc. each).

1.	Pigmentation changes	7
2.	Skin shedding	9
3.	Shelf reduction	10
4.	Gill reduction	14
5.	Upper lip fold reduction	33
6.	Tail fin reduction	39

The animal was alive at the termination of the study. The gold flecks were arranged in definite circles and a few were confluent. The shelves and gills were about one-half their original size. The upper lip folds were reduced anteriorly, and tail fin atrophy was complete.

Animal 55 received seven injections (1/2 cc. each).

1.	Pigmentation changes	6
2.	Shelf reduction	11
3.	Skin shedding	11

Death occurred 12 days subsequent to the first injection. The flecks had increased in number and were arranged in circles, none were confluent. The shelves had reduced slightly.

Specimen 48 received nine injections (1/2 cc. each).

1.	Skin shedding	4
2.	Pigmentation changes	9
3.	Tail fin reduction	11
4.	Gill reduction	15
5.	Shelf reduction	18
6.	Eye bulging	25
7.	Upper lip fold reduction	25
8.	Lower lip fold reduction	36

Death ensued 78 days after treatment. The flecks were numerous on the dorsal surface. They were arranged in definite circles, except on the head, where they were confluent and appeared as rings. The dorsal tail fin was completely reduced, and the gills were approximately one-fourth their original length. The shelves had reduced about 75 percent. The upper lip folds were gone, and the

lower lip folds appeared as a thin, dark line. The eyes bulged about one-half as much as the adult state.

Animal 28 received 38 injections (1/2 cc. each). Six were of Rana and the remainder were of Taricha pituitary homogenate.

1.	Pigmentation changes	23
2.	Skin shedding	26
3.	Gular fold fusion	26
4.	Shelf reduction	26
5.	Upper lip fold reduction	27
6.	Eye bulging	28
7.	Lower lip fold reduction	37

The specimen died 48 days following the first injection. Pigmentation changes took place on the head and near the base of the dorsal tail fin. The gold flecks increased in number, assumed the circular arrangement, and became confluent. Elsewhere on the dorsal surface, they were sparse and arranged in no definite pattern. Gular fold fusion was complete, except for a small region adjacent to each gill. The dorsal tail fin and gills showed no reduction. The shelves and upper lip folds were gone, while the lower lip folds appeared as thin, dark lines. Eye bulging had progressed to approximately three-fourths that of the adult state.

Neotene 171 received four injections (1 cc. each) of homogenate prepared from its own gland and from those of other neotenes (174, 176, 181). The animal was alive when the study was terminated. It exhibited no atrophy of larval structures, but did utilize the gravel.

It was first seen out of the water on the tenth day following treatment, and has been observed on the gravel for as long as 12 hours.

All of the neotenes, which received pituitary homogenate, shed small, torn fragments of skin. Sloughing was continuous once it started. Moreover, they utilized the gravel for long periods of time and assumed the characteristic rigid stance.

DISCUSSION AND CONCLUSIONS

Normal Metamorphosis

1. Length and time of year at metamorphosis. Kessel and Kessel (1944) reported that transformation occurs when the larvae are between 130 and 140 millimeters in total length. They found no metamorphosing individuals and based their conclusion on the specimens collected June 21 and July 21, 1942. Five second-year larvae were taken on June 21. They measured 125, (sic) 134, 136, and 141 millimeters, giving an average length of 135. 2 millimeters. On July 21, five second-year larvae, whose individual lengths were 120, 132, 136, 138, and 140 millimeters, were secured. These had an average length of 133. 2 millimeters, two millimeters less than those collected the preceding month. Fifty-five percent of the animals captured June 21 were second-year larvae, while on July 21 only 19 percent belonged to this class. Therefore, they thought that, ". . . during the month between June 21 and July 21 many of the second-year larvae had metamorphosed and forsaken the creek habitat for the terrestrial one of their adult state."

Their collection, made on August 21, revealed a further decrease in average length and also in the number of larvae. Four second-year specimens were taken, having an average length of 131 millimeters. This was 1. 2 millimeters less than that of the

previous month.

No second-year larvae were found on September 21. Thus, they asserted that metamorphosis ". . . normally begins in the early summer and continues until about the end of September."

During the present study, three larvae, taken on June 19 (2) and August 24 (1), were transforming when collected. Their individual total lengths were 111.25, 120.05 and 127.75 millimeters.

Three small adults, having total lengths of 111.50, 111.50, and 117.10 millimeters, were secured, two on August 14, and one on July 23 respectively. They were in shallow water near the edge of the stream. It was assumed that they had just completed the change from larval to adult life.

Furthermore, four adults, one obtained the 14th, and three the 15th of March, had total lengths of 108, 128, 133 and 155 millimeters respectively. They were under damp moss near the stream edge. If one assumed that they transformed the preceeding summer, the three smaller specimens must have been abnormally small when the change occurred. Unfortunately, nothing is known about the rate of growth of the adult form.

All of the above animals, except the 117.10 and 127.75 millimeter larvae, came from Tillamook County (see Appendix). This area is considered to be the middle portion of the Oregon coastal mountains. Reed (1949) states that neither larvae or adults,

larger than approximately 110 millimeters in total length, have been taken from this locality.

This investigation, however, revealed that larger individuals, both larvae and adults, inhabit the middle portion of the Oregon coastal mountains. The following specimens were taken: numbers 22, 23, 24, 25, 34, 48, 49, 50, 51, 52, 55, 57, 59, 71, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 186, 187, 188, 189, and 190. It should be noted that some of the larvae were exceptionally large, approaching the size of the 286 millimeter specimen mentioned by Bishop (1943).

All of the Dicamptodon which were transforming when collected, and those which were assumed to have metamorphosed shortly before they were taken, were smaller than the lower limit proposed by Kessel and Kessel (1944) for the normal length at metamorphosis (130 millimeters).

If one considers the animals that transformed in the laboratory, both at room temperature and at 4^{o} Centrigrade, the upper limit, hypothesized by the above authors (140 millimeters), may be extended. The largest specimen showing transformation changes was 166.25 millimeters in total length. Larvae having total lengths of 141.35, 143.00, 144.55, 147.35, 148.25, 149.40, 156.10 and 159.55 millimeters exhibited metamorphic changes.

In view of the above findings, the writer concludes that larvae,

in Oregon, are capable of transforming when they are between approximately 111 and 166 millimeters in total length. On the other hand, the smallest transformed individual was the 96.5 millimeter specimen mentioned by Reed (1949). It was taken on April 13, 1946. Kessel and Kessel (1944) obtained an adult measuring 105 millimeters in total length, but it was not considered in determining the normal size range at metamorphosis, as they assumed it to be a stunted individual (consult page 1). This specimen was abnormally small, at the time of transformation, if one considers only their findings. It should be recalled, however, that they found no transforming individuals and based their conclusions on a growth study in which they obtained small numbers of second-year larvae, five in June, five in July, and four in August. Furthermore, the animals were not marked, so the above authors could not be certain that recaptures were obtained. In light of the present investigation, the 105 millimeter specimen does not seem so abnormally small at the time of transformation. The fact that it lacked a hind leg does not account for its size, as these animals are extremely cannibalistic. In addition, three small adults, contained in the collection of the Oregon State Museum of Natural History, have been taken in Oregon; (OSUMNH-numbers, 786, 4239, 5250). They measured 104, 102, and 104 millimeters, respectively, in total length.

The 96.5 millimeter adult reported by Reed (1949) was collected

about three months prior to the date at which Kessel and Kessel (1944) first obtained data indicating that metamorphosis was taking place. A small adult, 101 millimeters long, was taken on March 23, 1955, at Prairie Creek State Park, Humbolt County, California, by D. Ferguson. Another adult, 128. 20 millimeters in total length, was obtained on May 14, 1957, alongside Oregon highway 34, 20 miles west of Alsea, Lincoln County, Oregon. The above data indicate that transformation may begin in the spring. Observations in the laboratory also support this hypothesis. A second-year larvae was taken from Hyde Creek, approximately 7-1/2 miles southwest of Philomath, Benton County, Oregon, on February 26, 1965. It measured 122. 85 millimeters in total length. The writer, at this time, was not familiar with the more subtle indications of transformation. It was not observed daily, as attempts to capture specimens were underway and much time was spent in the field. Thus, it is not known when transformation began, but metamorphosis was completed by March 20. The data on normal metamorphosis revealed that the process required approximately 28 days. Therefore, it is possible that the larva was in its initial phase of transformation when collected. The fact that this animal metamorphosed in the spring, might account for small adults taken at this time of the year.

2. Sequence of events and their time relationships. Kessel and Kessel (1944) observed two second-year larvae during normal

metamorphosis. The writer thinks that their observations were superficial, as nothing appears in their publication concerning the atrophy of the prominent lip folds, the bulging of the eyes, the fusion of the gular fold, the formation of the eye-stripe depression, or the formation of the adult snout.

That a comparison may be made, their observations are given in some detail:

Specimen A, having a total length of 130 millimeters, showed tail fin and gill atrophy on July 15, while specimen B exhibited gill reduction on August 10. Tail fin abatement began two days later.

The present study, however, has revealed that five events began prior to initial gill reduction: pigmentation changes, shelf reduction, eye bulging, tail fin atrophy, and upper lip fold reduction. The tail fin began atrophy approximately three days prior to gill reduction, which occurred nine to ten days after metamorphosis was initiated.

The gills of specimens A and B appeared as stumps, having filaments about one-fifth their original length, nine and ten days respectively, after initial reduction. Gill atrophy required 11 days for specimen A and 18 days for specimen B, becoming complete at the time of the final molt.

The animals observed during this investigation reached the above-mentioned stage approximately four days after initial gill reduction. Five to six days later, the gills appeared as small pigmented

masses and in three to five days they were completely absorbed. This was at the time of, or shortly after, the final molt. Therefore, gill reduction required 12 to 15 days. Kessel and Kessel do not mention the pigmented masses.

The above mentioned authors noted that neither larva completely exposed its gills to the air until the branchial filaments were about one-fifth their normal length. Even then, they were not allowed to become dried.

It has been the writer's experience that the larvae would leave the water shortly after initial shelf reduction, before any indication of gill atrophy, for extended periods of time (see page 27).

Kessel and Kessel use the final molt, which occurred 11 and 18 days respectively after the beginning of gill reduction, as the criterion of metamorphosis. No sloughing prior to the final molt was mentioned. Reading their publication, one cannot help but think that the marbled pattern of the adult became evident immediately after the integument was shed; "This revealed the marbled markings of the adult salamander."

This investigation showed that the final molt, beginning about 12 days after initial gill reduction, did not mark the end of the transformation process. Two events occurred subsequent to the shedding of the larval skin: (1) The eyes made their final protrusion and, (2) the dorsal depression was invaded by gold pigment. The black

pigment was either completely replaced or remained as a thin, dark line. The adult form was attained 18 to 19 days after the gills began to atrophy. If one considered the final molt as the criterion of metamorphosis, the time required for the process agreed well with specimen A but was relatively short in comparison to specimen B.

In the present study, all ten animals sloughed integument prior to the final molt. They shed small fragments approximately two days after the branchiae began to abate. Furthermore, six of the larvae shed larger pieces of skin seven to nine days subsequent to initial gill reduction. This was three to five days before the final molt. Four of the animals failed to exhibit the second molt.

Pigmentation changes of the integument marked the beginning of metamorphosis. The change from the more or less dark, uniform coloration of the larvae, to the brilliant gold and black marbled pattern of the adult, is a gradual process. The specimens appeared brighter just after the final molt. The marbled pattern, however, was evident, to the unaided eye, before this event occurred.

Metamorphosis at 4° Centigrade

It is well established that cold blocks the transformation of certain urodeles (Lynn and Wachowski, 1951; Noble, 1931). Neotenes of Ambystoma mexicanum, and Ambystoma tigrinum are found at high elevations, while members of the species at low elevations

exhibit a normal metamorphosis. Neotenes of Ambystoma gracile, which are more abundant at high elevations, are also found at low elevations. Snyder (1956) reports metamorphic retardation of an unknown percentage of larvae living at sea level. Moreover, neotenous individuals of Ambystoma gracile inhabit Owl Creek, Linn County, which is on the floor of the Willamette Valley.

Snyder (1956) ascertained evidence in support of the hypothesis that there is a high proportion of metamorphic failure in montane urodeles. He maintained larvae (Ambystoma gracile) in thyroxine solution (1/500, 000 concentration) at 34°F and they failed to respond.

Dicamptodon larvae will respond at 4° Centigrade (39. 2° Fahrenheit), but it is realized that this temperature is 5. 2 degrees higher than that reported by Snyder to block transformation. Whether this difference would enable the former species to metamorphose is not known. All 11 of the Dicamptodon responded within a week after they were placed in the refrigerator. Eight of them died when transformation was about three-fourths completed, but three completed metamorphosis. Thus, transformation was not blocked, although it was slowed down considerably, requiring approximately 146 days. The fact that the majority of the animals died before completing transformation does not suggest that a larger number of neotenes may be found at higher elevations. This would have been indicated if the larvae had not started metamorphosis.

Both adults and larvae (Dicamptodon) have been taken from the higher elevations (up to 5, 000 feet). In fact, after reviewing the specimens which are available in the collection of the Oregon State Museum of Natural History, the writer found that more neotenes have been collected at lower elevations than from higher elevations. The converse holds true for adults. This, however, could be due to the choice of collecting sites and the method employed to obtain the animals. The writer feels that efforts to collect neotenes of this species from the higher elevations have been limited. The data indicate that low temperature is not a feasible explanation for the metamorphic failure of Dicamptodon ensatus.

Larvae Denied Shallow Water

Kessel and Kessel (1944) deprived two animals the opportunity to leave the water. Specimens C and D were maintained in 2-1/2 and 5 inches of water respectively. They remained in a partial state of transformation until the water level was lowered. Their transformation is given in detail so that a comparison may be made with the data of the present study:

Metamorphic changes were manifested in specimen C on August 20 when it had attained a total length of 135 millimeters. The branchial filaments were reducing, and caudal fin atrophy was evident. The animal stood on its hind legs all the time, as this was the only

way it could reach the surface and expose its snout. Skin shedding began on August 23. By August 25 the gills were about one-half their original size. The tail fin had also disappeared. Up until this point, metamorphosis had progressed normally, but as long as the animal was left in deep water, no further changes occurred. It remained in this state over six months. Late in February the water level was lowered and within a week transformation was completed.

Specimen D began transforming on September 22 when it was 140 millimeters long. At this time skin began to slough, revealing the marbled markings of the head. No further structural change took place until the last part of November. The salamander made numerous attempts to get its snout above water. It finally achieved this by balancing on the tail, and transformation then proceeded to the point where the gill filaments were greatly reduced and the caudal fin had disappeared. As long as the animal was denied shallow water, no further changes occurred. In late February the water level was lowered, and within a few days transformation was completed.

They concluded that the inability to emerge causes a physiological block to the completion of metamorphosis.

During the present investigation, 12 larvae were maintained in five inches of water. Nine of the animals died before any indication of transformation. Two showed no metamorphic changes and were alive at the termination of the study. One larva did transform (see

pages 29-32). Although its metamorphosis differed considerably from the normal metamorphosis of the species, the onset of the process was not delayed as in Kessel's specimens. Constriction of the snout occurred seven days after the animal was placed in deep water. None of the animals which underwent normal metamorphosis exhibited initial shelf reduction prior to pigmentation changes. Pigmentation changes and eye bulging were manifested six days later. Moreover, the gills began their reduction relatively late. By this time the snout and the pigmentation resembled that of the adult. The tail fin was evident until the 34th day of transformation. Three molts occurred, the final one of the 62nd day. Up until this point, the animal had made no attempt to surface. The following day, however, continuous attempts to emerge were observed and the water level was then lowered. Within three days, the eyes made their final protrusion. Transformation was completed in 67 days. It required 39 days longer than the larvae which metamorphosed in shallow water at room temperature.

In light of the above observations and in relation to the streams where neotenes were collected, the writer questions the validity of the "deep water" hypothesis. Two larvae did not transform while in deep water. This, however, does not necessarily indicate the deep water prevented their transformation because one metamorphosed under such conditions. Furthermore, three larvae,

maintained in shallow water, failed to transform. In nature, these individuals might have become neotenes.

Induced Metamorphosis

1. Reaction of nonbreeding larvae to L-thyroxine injections. This aspect of the investigation was conducted so that the writer, after observing the metamorphic changes, could more accurately analyze the normal transformation of the species. Since there were many variables, such as lengths and ages of specimens, and dose and frequency of injections, the metamorphic changes of these nonbreeding larvae will not be compared with those of the larvae which underwent normal metamorphosis.

It should be noted, however, that one animal (32) exhibited snout changes subsequent to pigmentation changes, the converse being the case with the other larvae. Moreover, due to its small size, 85.5 millimeters total length, the writer believed that it was of the first-year age class.

None of the specimens could be carried completely through metamorphosis, although four reached the point where the gills were small, pigmented masses. For a general description of their responses consult pages 32, 33 and 34.

2. Reaction of second-year larvae to thyroid homogenate injections. Two second-year larvae were injected with thyroid

homogenate prepared from the glands of neotenes. In this manner it could be determined whether or not the neotenous thyroid contained a sufficient amount of colloid to induce metamorphosis. Both animals responded. One received 0. 812 of the gland and showed pigmentation changes after an eight day latent period. On the ninth day, the shelves began to atrophy. Death occurred 13 days after treatment.

The other larvae was administered 0. 375 of the gland and responded with pigmentation changes five days subsequent to treatment. The shelves began to reduce on the ninth day. It was found dead in the water 57 days after the first injection. Except for two small, pigmented gill-masses, two small areas of the gular fold which were not fused, and bulging of the eyes three-fourths that of the adult state, the animal appeared completely metamorphosed.

It was concluded that the neotenous thyroid contains a sufficient amount of colloid to induce transformation in second-year larvae.

3. Reaction of neotenes to L-thyroxine injections. Two neotenes were injected with thyroxine to determine if their tissues were insensitive; both responded to the treatment. Shelf reduction occurred two days prior to pigmentation changes and gill atrophy, which took place simultaneously. They died in the terminal stage of metamorphosis when the gills appeared as small, pigmented masses. The dark mottling had just become evident, the eyes bulged considerably, the snout appeared as that of the adult, and tail fin reduction was

complete.

The above data indicate that neotenes are capable of responding to thyroxine treatment, and it is not felt that tissue insensitivity plays a major role in the metamorphic failure of this species.

4. Reaction of neotenes to thyroid homogenate injections. Since metamorphosis was induced in neotenes, treated with L-thyroxine, and in second-year larvae, injected with neotenous thyroid homogenate, it was imperative that neotenes be tested for response to the product of their own gland.

Swingle (1924) conducted experiments in which he removed the entire thyroid glands from neotenic Ambystoma tigrinum. The thyroid from each animal, plus those from two other neotenes, was reinjected. Two larvae metamorphosed but six did not. He states that metamorphosis occurred if the neotenes were fed thyroid substance or injected with thyroxine or other iodine compounds. Moreover, he ascertained that if the thyroid was cut into pieces and injected into thyroidless anuran tadpoles, they transformed. As many as 13 tadpoles metamorphosed under the influence of one gland. Swingle concluded that, "Other factors besides the thyroid are involved in axolotl neoteny-factors involving sensitization of the tissues to the hormone."

Earlier, however, Swingle (1922) had asserted that, "the failure of the axolotl to metamorphose appears to be due to the inhibition or

the defective development of some unknown factor which normally serves to release the fully formed hormone from the thyroid into the bloodstream. "

Subsequent to Swingle's work, many investigators have adduced evidence that the releasing factor is produced by the pars anterior of the pituitary (Allen, 1932; Blount and Blount, 1947; Blount, 1950; Burns, 1930; Grant, 1931; Ingram, 1929).

The present study revealed that neotenes will respond to the product of their own thyroid (see page 39). Metamorphosis, however, was not completed. Animal 48 showed the highest degree of transformation, and was alive at the termination of the study. The gills were approximately one-fourth their original length; the snout appeared adult; the tail fin had completely atrophied, and the eyes bulged considerably. The gular fold had not fused, nor was the mottled pattern evident.

5. Reaction of neotenes to pituitary homogenate injections. Neotenes were tested for reactions to ranid and salamandrid pituitary homogenate which was prepared from glands assumed to be normal in respect to their production of the releasing factor.

Ten animals were treated, each receiving the equivalent of one to 19 glands (refer to pages 40-45). Transformation was induced in each animal, although none could be carried through to the adult state. It was possible, however, to attain approximately the same degree

of metamorphosis as was achieved by injecting neotenes with their own thyroid homogenate. In fact, one specimen showed partial fusion of the gular fold. Therefore, neotenes are capable of responding to the product of "normal" pituitaries.

One animal received pituitary homogenate prepared from its own gland and from those of other neotenes. Over a seven month period, it exhibited no overt signs of metamorphosis. Although tentative, the data indicate that the Dicamptodon pituitary may not produce the releasing factor in sufficient quantity to induce the thyroid to release its hormone. In fact, there was no evidence that it was produced.

In brief conclusion, the data of this study concerning metamorphic failure is very tentative, as a limited number of neotenes could be obtained for experimentation. It does, however, indicate that low temperature, deep water, and tissue insensitivity play no major role in the metamorphic failure of this species. Rather, it is believed that there is some defect in the interrelation of the various components of the endocrine system, possibly the pituitary-thyroid complex. If this is the case, the next step would be to determine the factors responsible for this defect, whether they are hereditary, environmental or a combination of the two.

SUMMARY

This study began January 1965 and was terminated in March 1966. An intensive effort was made to obtain large numbers of animals, and 269 were collected. Four methods were employed to secure specimens: the line method, the hand method, the trapping method, and the road method. The small, nonbreeding larvae were more readily obtained than the large, sexually mature individuals, and adults were rarely taken. The method used determined the size of the specimens collected. All animals were kept in water which had been filtered through activated charcoal and crushed oyster shells.

The investigation was restricted by the small numbers of animals available for observation. Although a fair number were initially obtained, many died while in the laboratory. This resulted mainly from fouled water and cannibalism, but larvae also died from unknown causes.

Nonbreeding larvae were injected with L-thyroxine and observed daily for transformation changes. This experiment was conducted so that the writer would become familiar with the sequence of events and their time relationships, and could more accurately analyze the normal metamorphosis of the species.

Second-year larvae were observed during normal metamorphosis. The process was separated into eight stages: (1) pigmentation changes, (2) formation of the adult snout, (3) eye bulging,

(4) formation of adult tail, (5) gill reduction, (6) gular-fold fusion, (7) formation of eye-stripe depression, and (8) skin shedding. These were studied in relation to their sequence of occurrence and their time relationships.

Some second-year larvae were maintained at 4° Centigrade, while others were kept in deep water, at room temperature, to determine the effects of low temperature and water depth on transformation. Metamorphosis was altered, but it was not blocked.

Data pertinent to size, age, and time of year at transformation were obtained. Metamorphic changes were exhibited by second-year larvae, not subjected to thyroxine treatment, ranging from 111.50 to 166.25 millimeters in total length. Three were transforming when collected. One specimen transformed three months earlier than the time formerly proposed for the species. It is believed that metamorphosis begins in the early spring, rather than in the early summer, and continues until the end of August. First-year larvae showed no tendency to metamorphose.

The literature needs to be brought up to date in respect to the maximum size of neotenes. The largest specimen seems to be the 312 millimeter specimen taken by Storm (see page 35). In addition, nothing could be found in print concerning the minimum size of breeding larvae. A 208 millimeter neotene was collected during the present study (consult page 34).

Since neotenes responded positively to intraperitoneal injections of L-thyroxine and their own thyroid homogenate, it is thought that tissue insensitivity plays no major role in their metamorphic failure. Moreover, they showed metamorphic changes following treatment with ranid and salamandrid pituitary homogenate. Transformation could not be induced by injecting homogenate prepared from neotenic pituitaries. Although tentative, the data indicate that the releasing factor is not produced. The storage factor, however, must be present in sufficient quantity, for they responded to their own thyroid homogenate. Second-year larvae also exhibited transformation changes following injections of homogenate prepared from neotenic thyroids.

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APPENDICES

APPENDIX A

COLLECTION SITES

Berry Creek - About eight miles north of Corvallis, Benton County, Oregon.

Buzzard Creek - Approximately 17 miles east of Lowell, Lane County, Oregon, where the stream crosses Fall Creek Road.

(3-1/2 GLR) three and one-half miles up Gauldy Lookout Road - Small spring approximately three miles southeast of Hebo, Tillamook County, Oregon.

(6-1/2 GLR) six and one-half miles up Gauldy Lookout Road - Small stream about five miles southeast of Hebo, Tillamook County, Oregon.

(7-1/2 GLR) seven and one-half miles up Gauldy Lookout Road - Small stream approximately six miles southeast of Hebo, Tillamook County, Oregon.

(HMt.) Hebo Mountain - Alongside Hebo Mountain Road, approximately five miles east of Hebo, Tillamook County, Oregon.

(HRA) Hebo Recreation Area - Small stream about seven miles east of Hebo, Tillamook County, Oregon.

Hyde Creek - Approximately seven and one-half miles southwest of Philomath, Benton County, Oregon.

Mill Creek - One mile north of Logsden, Lincoln County, Oregon.

Nichols Creek - Four and one-half miles southwest of Monroe, Benton County, Oregon.

(N. Trail M. P.) North Trail Marys Peak - Small stream along the trail, about five miles south of Blodgett, Benton County, Oregon.

Oak Creek - Approximately three miles west of Corvallis, Benton County, Oregon.

Parker Creek - Near headwaters at Marys Peak Recreation Area, about 14 miles southwest of Corvallis, Benton County, Oregon.

Parker Creek Falls - Approximately four-tenths mile southwest of Marys Peak Summit.

Portland Creek - About 15 miles east of Lowell, Lane County, Oregon.

Scio-Hatchery RR- (Roaring River) - Approximately seven miles southeast of Scio, Linn County, Oregon.

Soap Creek - About five miles north of Corvallis, Benton County, Oregon.

(S. S. R. -Trout Creek) - South Santian River near Trout Creek Forest Camp, approximately eight miles east of Cascadia, Linn County, Oregon.

Sunshine Creek- One mile up He He Road, about 16 and one-half miles east of Lowell, Lane County, Oregon.

(TWC) Tributary of Woods Creek - Small stream flowing into Woods Creek, approximately three miles up Woods Creek Road near Starker Tree Farm.

Woods Creek - About one and one-half miles northwest of Philomath, Benton County, Oregon.

APPENDIX A

ANIMALS OBTAINED

No.	Date Collected	Where Collected	County	Total L.	S. V. L.	Form
1	23 Jan. 65	Berry Cr.	Benton	250.00	160.00	L
2	24 Jan. 65	Hyde Cr.	"	247.00	175.00	L
3	26 Feb. 65	Hyde Cr.	"	215.00	136.00	L
4	7 Mar. 65	Parker Cr.	"	110.00	64.00	L
5	"	Woods Cr.	"	186.00	121.00	L
6	"	TWC	"	155.00	100.00	L
7	9 Mar. 65	Hyde Cr.	"	151.00	87.00	L
8	"	"	"	136.00	80.00	L
9	"	Woods Cr.	"	260.00	161.00	A
10	"	"	"	208.00	122.00	L
11	"	Hyde Cr.	"	45.00	23.00	L
12	12 Mar. 65	TWC	"	130.00	80.00	L
13	"	"	"	126.00	77.50	L
14	"	"	"	117.00	71.00	L
15	"	"	"	142.00	80.00	L
16	"	"	"	161.50	97.00	L
17	"	Woods Cr.	"	237.00	153.00	L
18	"	Hyde Cr.	"	122.80	77.00	L
19	14 Mar. 65	3-1/2 GLR	Tillamook	38.50	20.10	L
20	"	7-1/2 GLR	"	108.00	65.00	A
21	"	3-1/2 GLR	"	48.00	25.00	L
22	15 Mar. 65	7-1/2 GLR	"	128.00	77.00	A
23	"	7-1/2 GLR	"	133.00	81.00	A
24	"	"	"	155.00	97.00	A
25	"	HRA	"	137.00	86.00	L
26	"	"	"	101.00	60.00	L
27	23 Mar. 65	Hyde Cr.	Benton	117.00	73.00	L
28	"	Woods Cr.	"	215.00	132.00	L
29	27 Mar. 65	"	"	237.50	150.00	L
30	4 May 65	"	"	285.00	174.00	A
31	12 May 65	Oak Creek	"	264.00	170.00	L
32	"	Nichols Cr.	"	85.00	50.00	L
33	18 May 65	Woods Cr.	"	243.00	151.00	L
34	22 May 65	HMt.	"	265.00	163.00	A
35	23 May 65	Parker Cr. Falls	"	182.00	113.00	A
36	27 May 65	Parker Cr.	"	111.15	69.30	L
37	"	"	"	88.59	57.10	L

No.	Date Collected	Where Collected	County	Total L.	S. V. L.	Form
38	27 May 65	Parker Cr.	Benton	84. 30	58. 15	L
39	"	"	"	236. 00	149. 00	L
40	"	"	"	140. 00	86. 00	L
41	"	"	"	117. 50	72. 00	L
42	"	"	"	139. 00	90. 00	L
43	"	"	"	107. 50	65. 00	L
44	9 June 65	Mill Cr.	Lincoln	56. 00	32. 00	L
45	"	"	"	54. 00	29. 50	L
46	"	"	"	55. 50	30. 50	L
47	"	"	"	53. 00	30. 00	L
48	"	"	"	285. 00	187. 00	L
49	"	"	"	280. 00	176. 00	L
50	"	"	"	250. 00	177. 00	L
51	"	"	"	285. 00	183. 00	L
52	"	"	"	236. 00	171. 00	L
53	19 June 65	6-1/2 GLR	Tillamook	75. 70	42. 05	L
54	"	"	"	81. 80	50. 45	L
55	"	"	"	209. 00	131. 50	L
56	"	"	"	80. 15	46. 20	L
57	"	"	"	112. 20	67. 40	L
58	"	"	"	95. 20	57. 35	L
59	"	"	"	111. 25	69. 00	T
60	"	"	"	82. 85	46. 60	L
61	"	"	"	91. 80	51. 85	L
62	"	"	"	104. 60	58. 90	L
63	"	"	"	82. 05	51. 40	L
64	"	"	"	93. 90	51. 00	L
65	"	"	"	83. 90	47. 20	L
66	"	"	"	69. 25	39. 85	L
67	"	"	"	87. 07	49. 60	L
68	"	"	"	91. 05	53. 45	L
69	"	"	"	67. 35	37. 45	L
70	"	"	"	82. 50	48. 40	L
71	"	3-1/2 GLR	"	120. 05	73. 60	T
72	3 July 65	Parker Cr.	Benton	113. 90	68. 20	L
73	"	"	"	93. 50	52. 35	L
74	"	"	"	61. 80	34. 70	L
75	"	"	"	44. 40	23. 90	L
76	"	"	"	124. 25	74. 25	L
77	"	"	"	108. 30	63. 00	L
78	5 July 65	"	"	249. 00	158. 10	A
79	"	"	"	60. 50	33. 20	L
80	"	"	"	41. 30	21. 95	L

No.	Date Collected	Where Collected	County	Total L.	S. V. L.	Form
81	5 July 65	Parker Cr.	Benton	55.50	29.00	L
82	"	"	"	85.60	52.05	L
83	"	"	"	65.80	37.95	L
84	"	"	"	110.05	71.95	L
85	"	"	"	80.65	46.30	L
86	"	"	"	142.90	81.10	L
87	"	"	"	79.70	46.70	L
88	"	"	"	81.35	46.10	L
89	"	"	"	76.85	48.20	L
90	"	"	"	92.95	54.20	L
91	"	"	"	91.00	51.50	L
92	"	"	"	61.40	34.00	L
93	"	"	"	95.80	54.80	L
94	"	"	"	80.10	48.00	L
95	"	"	"	86.80	51.85	L
96	"	"	"	114.60	75.15	L
97	"	"	"	86.50	49.00	L
98	"	"	"	133.60	81.35	L
99	"	"	"	96.40	55.35	L
100	"	"	"	160.35	92.10	L
101	"	"	"	110.90	70.50	L
102	"	"	"	127.10	75.55	L
103	"	"	"	108.65	70.00	L
104	"	"	"	134.30	83.10	L
105	"	"	"	101.00	57.50	L
106	"	"	"	125.30	76.45	L
107	"	"	"	111.75	74.50	L
108	"	"	"	134.30	79.55	L
109	"	"	"	100.00	64.50	L
110	"	"	"	132.50	76.50	L
111	"	"	"	109.40	64.60	L
112	"	"	"	143.35	86.50	L
113	"	"	"	115.60	70.75	L
114	"	"	"	132.05	84.30	L
115	"	"	"	124.30	71.70	L
116	"	"	"	114.95	73.40	L
117	"	"	"	93.15	52.40	L
118	"	"	"	105.20	63.45	L
119	"	"	"	92.80	54.95	L
120	"	"	"	111.35	66.40	L
121	"	"	"	96.50	56.45	L
122	"	"	"	127.90	82.35	L
123	"	"	"	138.40	88.70	L

No.	Date Collected	Where Collected	County	Total L.	S. V. L.	Form
124	5 July 65	Parker Cr.	Benton	148.05	91.07	L
125	"	"	"	98.40	55.00	L
126	"	"	"	156.10	90.40	L
127	"	"	"	56.25	32.80	L
128	"	"	"	51.75	30.40	L
129	"	"	"	40.00	22.60	L
130	"	"	"	42.45	23.40	L
131	8 July 65	Parker Cr.	Benton	117.00	67.50	L
132	"	"	"	165.50	100.10	L
133	"	"	"	139.30	79.15	L
134	"	"	"	126.45	77.05	L
135	"	"	"	149.40	91.40	L
136	"	"	"	143.00	85.70	L
137	"	"	"	148.25	86.60	L
138	"	"	"	159.55	93.65	L
139	"	"	"	108.90	65.85	L
140	"	"	"	118.30	71.80	L
141	"	"	"	147.35	91.25	L
142	"	"	"	135.95	84.40	L
143	"	"	"	166.25	96.00	L
144	"	"	"	141.00	99.10	L
145	"	"	"	131.85	82.90	L
146	"	"	"	108.70	74.15	L
147	"	"	"	129.50	77.40	L
148	"	"	"	127.40	78.45	L
149	"	"	"	124.60	82.45	L
150	"	"	"	130.00	75.45	L
151	"	"	"	99.60	58.10	L
152	"	"	"	136.00	81.80	L
153	"	"	"	94.25	55.95	L
154	"	"	"	128.85	75.50	L
155	"	"	"	94.10	51.70	L
156	"	"	"	126.80	82.50	L
157	"	"	"	115.45	64.50	L
158	"	"	"	144.55	88.10	L
159	"	"	"	104.50	60.80	L
160	"	"	"	88.55	53.10	L
161	"	"	"	95.60	53.30	L
162	"	"	"	85.00	53.90	L
163	"	"	"	65.05	35.20	L
164	"	"	"	70.25	40.40	L
165	"	"	"	64.85	34.85	L

No.	Date Collected	Where Collected	County	Total L.	S. V. L.	Form
166	8 July 65	Parker Cr.	Benton	65.50	36.90	L
167	"	"	"	56.90	31.35	L
168	"	"	"	69.25	37.40	L
169	"	"	"	250.00	155.10	A
170	23 July 65	Parker Cr. Falls	"	117.10	75.40	A
171	29 July 65	Woods Cr.	"	292.00	191.00	L
172	31 July 65	S. S. R. -Trout Cr.	Linn	270.00	160.80	L
173	3 Aug. 65	Woods Cr.	Benton	155.00	92.00	L
174	4 Aug. 65	Mill Cr.	Lincoln	272.00	179.10	L
175	"	"	"	273.00	177.00	L
176	"	"	"	280.00	175.70	L
177	"	"	"	282.00	181.00	L
178	"	"	"	262.00	170.40	L
179	"	"	"	253.00	173.00	L
180	"	"	"	265.00	167.95	L
181	"	"	"	261.00	175.80	L
182	"	"	"	184.00	115.10	L
183	"	"	"	192.00	122.55	L
184	7 Aug. 65	N. Trail M. P.	Benton	190.00	117.00	A
185	11 Aug. 65	Soap Cr.	"	254.00	165.00	L
186	14 Aug. 65	6-1/2 GLR	Tillamook	111.50	66.50	A
187	"	"	"	222.30	142.15	A
188	"	"	"	111.50	64.50	A
189	"	"	"	116.50	70.35	L
190	"	"	"	117.15	70.20	L
191	"	"	"	109.50	65.20	L
192	"	"	"	94.30	54.00	L
193	"	"	"	91.15	52.45	L
194	"	"	"	89.85	51.65	L
195	"	"	"	83.05	51.00	L
196	"	"	"	96.90	57.54	L
197	"	"	"	97.00	59.30	L
198	"	"	"	99.30	56.00	L
199	"	"	"	82.70	54.90	L
200	"	"	"	99.35	61.50	L
201	"	"	"	86.75	50.00	L
202	"	"	"	90.05	52.40	L
203	"	"	"	89.50	52.20	L
204	"	"	"	94.25	54.90	L
205	"	"	"	89.74	52.40	L
206	"	"	"	98.10	56.10	L
207	"	"	"	90.65	52.10	L

No.	Date Collected	Where Collected	County	Total L.	S. V. L.	Form
208	14 Aug. 65	6-1/2 GLR	Tillamook	81.60	48.80	L
209	"	"	"	86.94	50.02	L
210	"	"	"	75.70	48.75	L
211	"	"	"	76.90	45.15	L
212	"	"	"	83.60	47.50	L
213	"	"	"	61.75	35.40	L
214	"	"	"	67.65	43.55	L
215	"	"	"	67.34	38.25	L
216	"	"	"	57.65	34.85	L
217	"	"	"	64.50	36.45	L
218	"	"	"	99.00	54.50	L
219	"	"	"	86.70	50.95	L
220	"	"	"	74.30	45.50	L
221	"	"	"	64.90	37.05	L
222	"	"	"	62.10	34.70	L
223	"	"	"	59.50	33.30	L
224	15 Aug. 65	N. Trail M.'s P.	Benton	236.00	149.10	A
225	17 Aug. 65	Scio-Hatchery RR	Linn	261.00	162.10	L
226	"	"	"	280.00	165.30	L
227	"	"	"	231.00	129.50	L
228	24 Aug. 65	Buzzard Cr.	Lane	127.75	77.10	T
229	"	"	"	69.00	40.00	L
230	"	"	"	76.35	43.35	L
231	"	"	"	52.15	31.00	L
232	"	"	"	53.70	30.25	L
233	"	"	"	48.30	30.00	L
234	"	"	"	55.90	31.20	L
235	"	"	"	45.90	28.40	L
236	"	"	"	55.00	31.25	L
237	"	"	"	53.05	30.00	L
238	"	"	"	55.50	32.60	L
239	"	"	"	56.50	33.00	L
240	"	"	"	52.95	32.00	L
241	"	"	"	54.90	31.20	L
242	"	"	"	54.00	31.45	L
243	"	"	"	55.60	31.35	L
244	"	"	"	49.35	29.30	L
245	"	"	"	47.30	29.40	L
246	"	"	"	70.90	38.90	L
247	"	"	"	62.20	41.25	L
248	"	"	"	84.90	52.50	L
249	"	"	"	65.90	42.00	L
250	"	"	"	67.50	38.70	L

No.	Date Collected	Where Collected	County	Total L.	S. V. L.	Form
251	24 Aug. 65	Buzzard Cr.	Lane	109.85	66.40	L
252	"	"	"	40.35	28.75	L
253	"	"	"	80.60	45.50	L
254	"	"	"	56.45	31.55	L
255	"	"	"	53.40	29.65	L
256	"	"	"	70.00	43.35	L
257	"	"	"	73.90	39.80	L
258	"	"	"	96.55	56.50	L
259	"	"	"	91.30	54.10	L
260	"	"	"	64.80	37.35	L
261	"	"	"	104.80	64.20	L
262	"	"	"	87.95	55.90	L
263	"	Portland Cr.	Lane	295.00	175.35	L
264	"	"	"	296.00	180.00	L
265	"	Sunshine Cr.	"	99.55	51.70	L
266	"	"	"	71.45	40.50	L
267	"	"	"	67.95	38.65	L
268	"	"	"	66.10	36.90	L
269	"	"	"	60.95	33.80	L

APPENDIX B
(Illustrations)

Figure 1.
Larva - lateral view

Legend:
A. Lower lip fold
B. Upper lip fold
C. Gular fold (unattached at midline)
D. Dorsal tail fin

Figure 2.
Larva - dorsal view

Legend:
A. Shelf
B. Yellow eye stripe
C. Dorsal depression

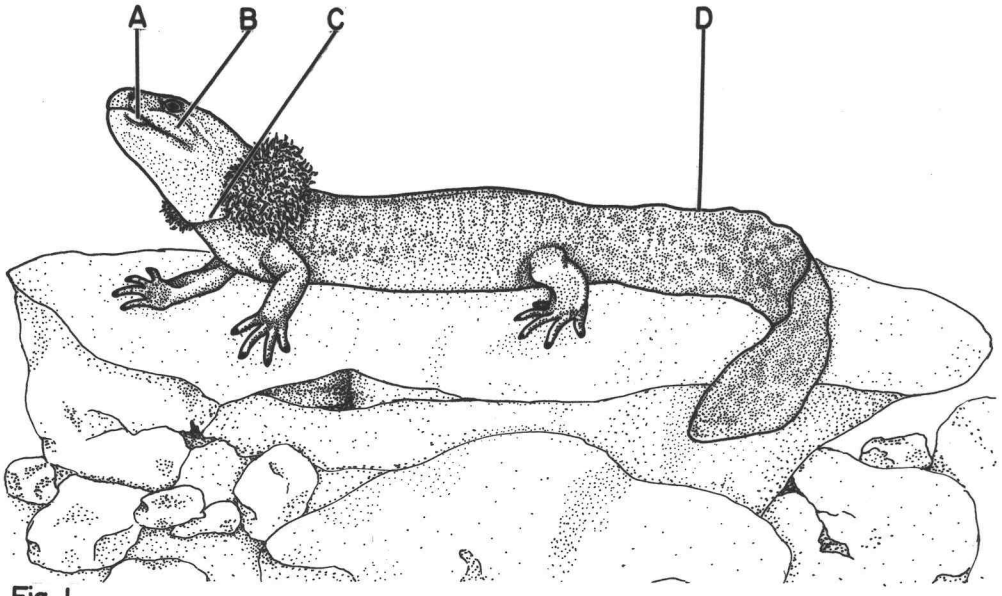

Fig. 1.

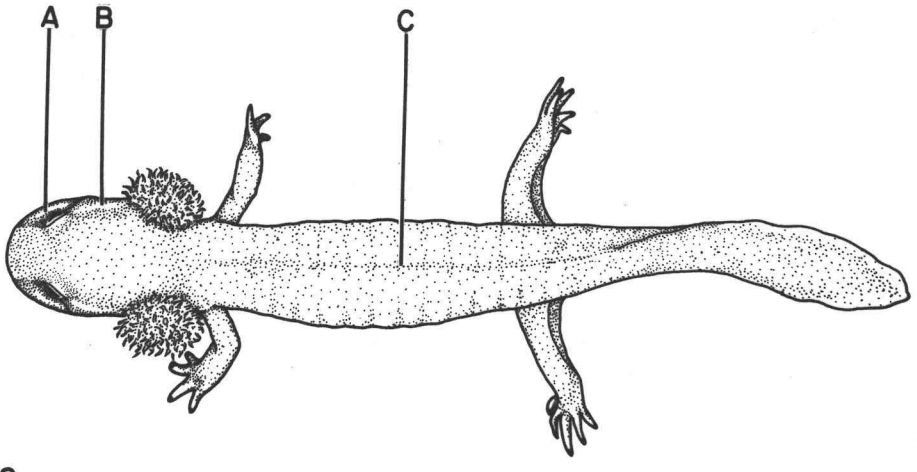

Fig. 2.

Figure 3.
The thyroid and the muscles of the ventral side of the head and throat.

Legend:
A. Intermandibularis (cut at the median raphe)
B. Geniohyoid
C. Branchiohyoid
D. Interhyoideus (cut at the median raphe)
E. Left thyroid mass
F. Insertion of the geniohyoid and rectus cervicis
G. Rectus cervicis

Figure 4.
Schematic drawing showing the gold flecks (A) scattered haphazardly over the dorsal surface in no particular pattern (see pages 22, 23).

Figure 5.
Schematic drawing showing gold flecks arranged in circles (A) (consult page 23).

Figure 6.
Schematic drawing showing the gold rings (A) which are formed when the flecks become confluent (see page 23).

Figure 7.
Schematic drawing showing the gold rings (A) after they have thickened (consult page 23).

Figures 8-11.
Atrophy of the dorsal tail fin (see page 25).

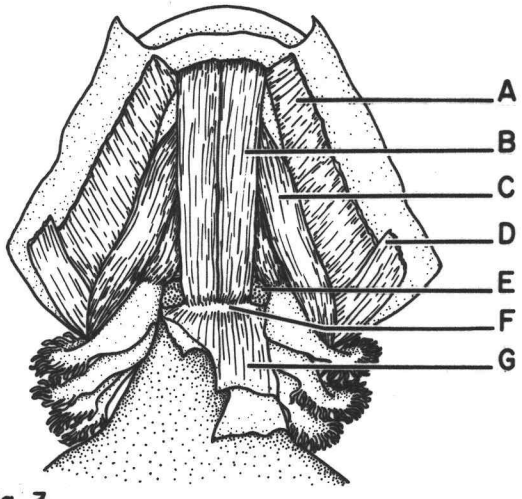

Fig. 3.

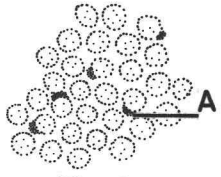

Fig. 4.

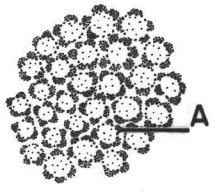

Fig. 5.

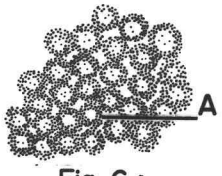

Fig. 6.

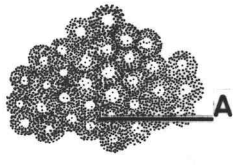

Fig. 7.

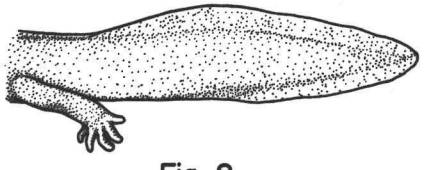

Fig. 8.

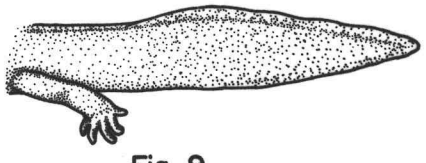

Fig. 9.

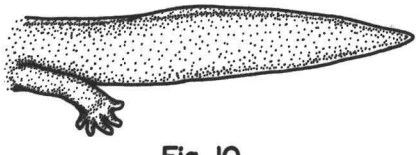

Fig. 10.

Fig. 11.

Figures 12-17.
These figures depict the various degrees of transformation in Dicampton. (Refer to pages 22-27).

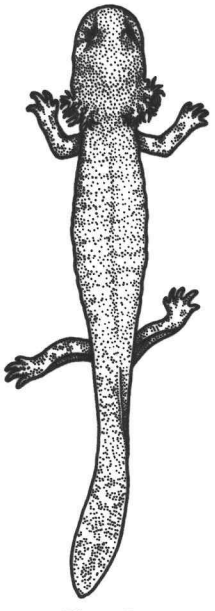

Fig. 12.

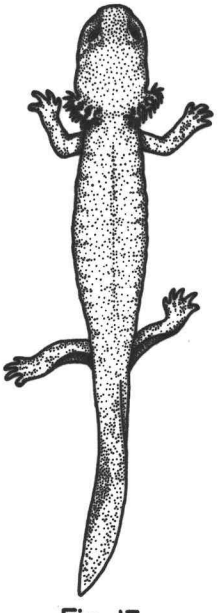

Fig. 13.

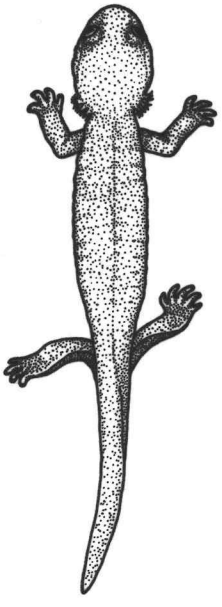

Fig. 14.

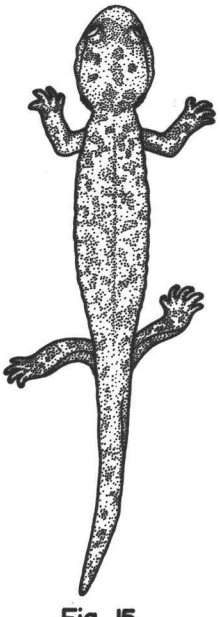

Fig. 15.

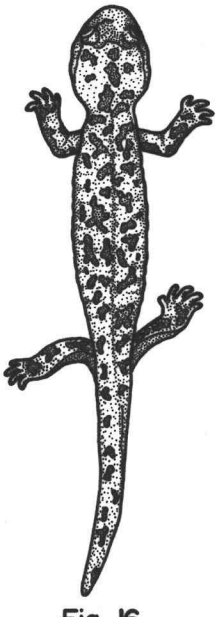

Fig. 16.

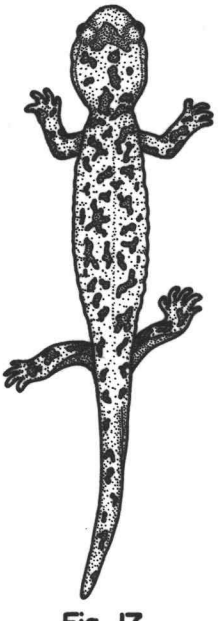

Fig. 17.

Figure 18.
Gular fold unattached at the midline (A).

Figure 19.
Gular fold attached at the midline (A).

Figure 20.
Gular fold fusion complete except adjacent to each pigmented gill mass (A).

Figure 21.
Gular fold fusion complete. A small gill mass (A) is present on each side of the neck.

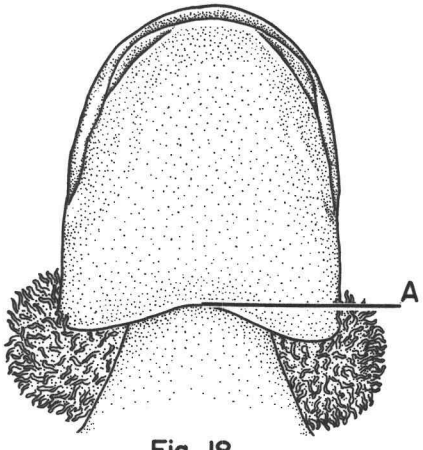

Fig. 18.

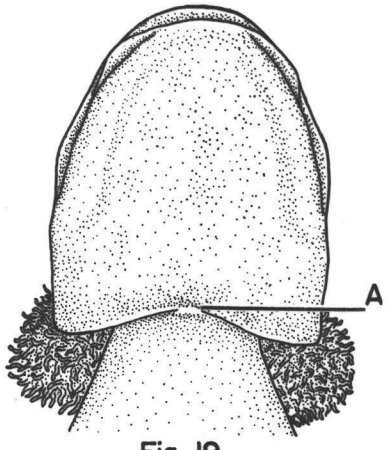

Fig. 19.

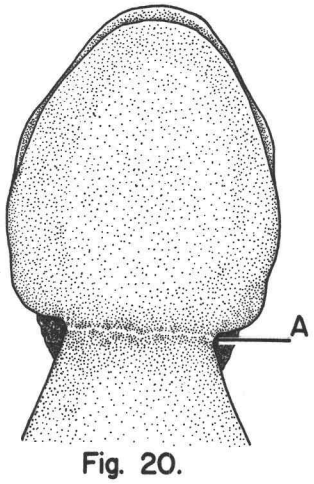

Fig. 20.

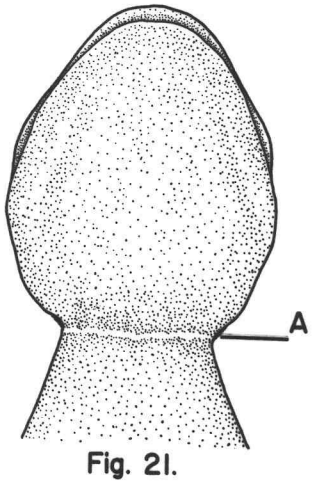

Fig. 21.

Figure 22.
Larva in advanced phase of metamorphosis.
The animal was transforming when collected.
(See page 28.)

Figure 23.
This illustration shows the greatest degree of transformation attained by neotenes which received thyroid and pituitary homogenate.

Figure 24.
Adult Dicamptodon.

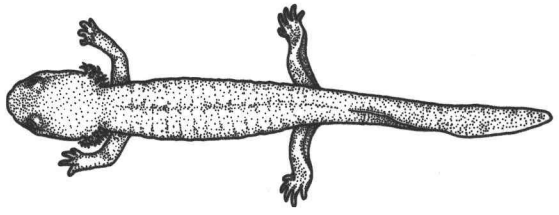

Fig. 22.

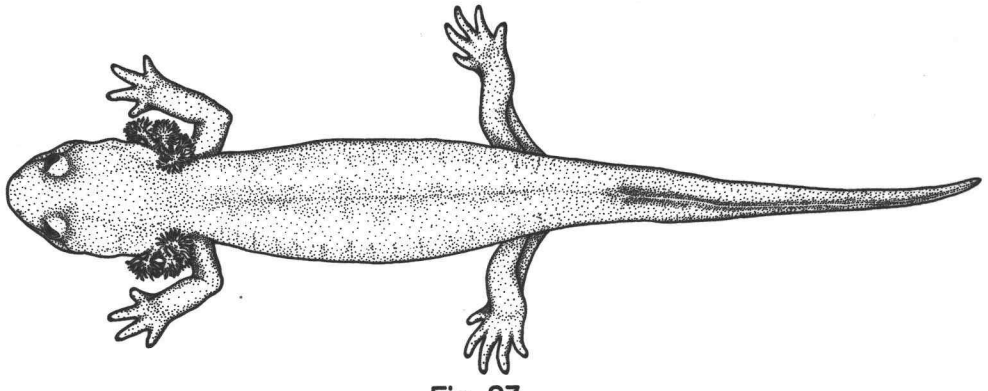

Fig. 23.

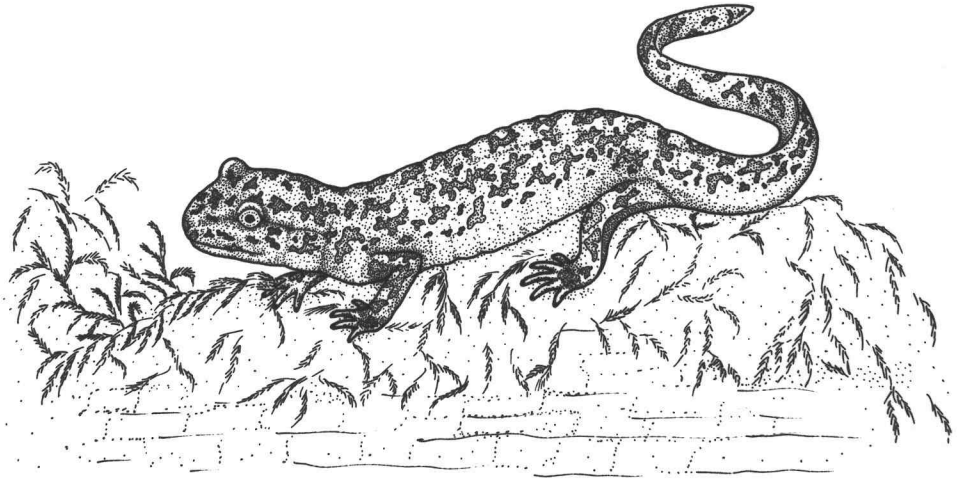

Fig. 24.