The thyroid gland of metamorphosing larval suckers Catostomus catostomus and Catostomus macrocheilus

Norman Sperry Tweed

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THE THYROID GLAND OF METAMORPHOSING LARVAL SUCKERS, CATOSTOMUS CATOSTOMUS AND CATOSTOMUS MACROCHEILUS

by

NORMAN S. THRED

B.S. Montana State University, 1962

Presented in partial fulfillment of the requirements for the degree of

Master of Arts

MONTANA STATE UNIVERSITY

1965

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Date
ACKNOWLEDGEMENTS

I am deeply grateful to Dr. George F. Weisel who provided the idea for this problem, offered many helpful suggestions throughout the study, and gave invaluable assistance in the preparation of the manuscript.

Support for this study was provided through a fellowship from the United States Department of Health, Education, and Welfare under authority of the National Defense Education Act.

Vital assistance in the photography was given by Dr. George W. Bartelmez, Mr. Earl Brandon, Mr. John Carpenter, and Dr. C. C. Gordon.

Numerous other faculty members, graduate students, and friends have provided helpful assistance and key ideas. Particular mention should be made of Dr. Ludvig G. Browman and Dr. Royal B. Brunson for key assistance. The chairman of the zoology department, Dr. Philip L. Wright, merits my thanks, also.

Particular recognition should be given to my mother, Mrs. H. O. Tweed, who provided help on the manuscript besides giving valuable inspiration.
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INTRODUCTION

GENERAL REMARKS

The phenomenon of metamorphosis in animals has intrigued biologists for many years. The marked morphological changes involved have been good targets for experimental studies, and the prospects for discovering its mechanisms have lured researchers into physiological and biochemical problems which have proved of value.

Metamorphosis has been defined by Etkin (1955) as "a definitely delimited period in postembryonic development during which marked developmental changes in non-reproductive structures occur." This event occurs less often among the fishes than among the amphibians. However, several species of fish (e.g., eels and flat-fishes) do undergo morphological changes shortly after hatching which warrant being called metamorphic. The degree of metamorphosis among fishes varies considerably. In some cases it is difficult to decide whether the change is metamorphic, or merely accelerated growth. The two closely related suckers used in this study, Catostomus catostomus (Forster) and Catostomus macrocheilus, Girard, undergo changes in morphology and behavior which are not so spectacular as, for example, those in the Pleuronectiformes, but sufficient to be termed metamorphic.

Because the thyroid gland has been shown to induce metamorphosis in amphibians, one might infer a similar function in teleosts. Much work has been undertaken in this direction, but the results have been far from conclusive. In fact, the data thus far collected have led
Gortman and Bern (1962) to state that "metamorphosis in fishes is probably relatively independent of the thyroid, and that thyroxine-induced metamorphosis is peculiar to the Amphibia." Barrington's (1963) remarks are similar, but somewhat more reserved, "Despite this evidence for the involvement of the thyroid hormones in the metamorphic changes of teleost fish, it would be unwise to look for too close a parallel with the situation in Amphibia." Thus, the thyroid gland may be involved in teleost metamorphosis, but much more information is needed to clarify the issue.

This study was undertaken to describe the anatomy of the thyroid gland of the sucker and to relate any histological changes in the gland to morphological changes in the fish during prometamorphosis, metamorphic climax, and postmetamorphosis. Histological changes in the thyroid have served as clues to its role in amphibian metamorphosis, and some of these clues are traced in this study.

LITERATURE REVIEW

Teleost Metamorphosis

Numerous species of teleosts exhibit peculiar morphological changes during their early development after hatching. The term metamorphosis defies precise definition, but is commonly applied to such phenomena in fishes. Barrington (1961) reviews the subject in this group of animals. Bertin (1957) reviews the literature describing the morphological metamorphosis of fishes, and suggests that the event is accompanied by a change in behavior. The extent of metamorphosis is greatest in fishes with pelagic larvae and benthic adults such as
in the Anguilliformes and Pleuronectiformes. Outstanding examples are the flatfishes in which the bilaterally symmetrical, pelagic larvae metamorphose into the familiar bottom dwelling, asymmetrical adult. Bertin's concept agrees with Wald (1958) as well as with Etkin (1955) who states, "The adaptive value of metamorphosis lies in the specialization of the larval form to one mode of life and of the adult to another."

With this in mind, the following observation by Stewart (1926) in regard to the white sucker, *Catostomus commersoni*, is quite interesting. He states, "As the mouth changes from a terminal to an inferior position, the fish must alter its diet, losing those forms that swim or float and sucking in the fauna of the stream bottom."

In his work on the early stages, he describes extensive changes in structures involved in feeding, especially the mouth which migrates from a terminal to a ventral position within a few weeks after hatching. Weisel (1960) has described morphological adaptations to bottom feeding in adult largescale suckers, *Catostomus macroleucus*. These include a ventral mouth with protractile apparatus, the development of which would require many osteogenic changes after hatching, inasmuch as newly hatched suckers have a terminal mouth. He has also described some adaptations to a microphagous diet in the adult longnose sucker, *Catostomus catostomus*, including the presence of a long, coiled stomachless gut (Weisel, 1962). The gut of early larval suckers is essentially a straight tube.
The Teleost Thyroid Gland

1. General Remarks

The first report on teleost thyroid glands was made by Simon (1844). He failed to find the gland in several species, presumably because his conclusions were based upon only gross observations. Later studies by Gudernatsch (1911) and others corrected his findings, and the thyroid gland is now generally considered to be present in all teleosts (Hoar, 1939). The extensive literature has been summarized in a number of reviews (Buddenbock, 1950; Lynn and Wachowski, 1951; Fontaine, 1953; Oliverou, 1954; Pickford, 1957; Vivien, 1958; Hoar, 1951, 1957, 1959; Berg, et al., 1959; Baggenstoss, 1960; Leloup and Fontaine, 1960; Corbin and Born, 1962; Corbin, 1963b; and Barrington, 1961, 1963). Use of radioiodine and other biochemical methods of evaluating thyroid activity in recent years has resulted in fewer contributions to detailed anatomical and histological descriptions. Numerous other reviews on various aspects of the vertebrate thyroid gland are available, (Barrington, 1933; Baggenstoss, 1939; Salter, 1940, 1950a,b; Fleischman, 1947, 1951; Grollman, 1947; New York Academy of Science, 1949; Barker, 1951; Roche, et al., 1953; Brookhaven Symposium on Biology, 1955; Corbin, 1955, 1959; Rawson, et al., 1955; Roche and Michel, 1955, 1956; Vanderlaan and Storrie, 1955; Warner, 1955; Gross, 1957; Ciba Foundation Colloquia in Endocrinology, 1957; Copenhagen and Johnson, 1958; Sonenberg, 1958; Pitt-Rivers and Tata, 1959; Roche, 1959; Desmarais, 1960; Fotherly, et al., 1960; Ingbar and Freinkel, 1960; Myant, 1960; New York Academy of Science Symposium, 1960; Solomon
2. Embryology

The earliest embryological study was by Maurer (1856) on trout. It is fairly well established that the thyroid gland of teleosts arises in a manner similar to most vertebrates. It begins at the level of the first and second visceral pouches as a median ventral downgrowth of pharyngeal epithelium (Pickford, 1957; Gorbman and Bern, 1962). One worker, however, has proposed a mesodermal origin for thyroid of two species of trout, Salmo irideus (S. gairdneri) and Salmo trutta (Thomopoulos, 1948, 1949). Pickford (1957) doubts this hypothesis. According to Gorbman and Bern, the thyroid anlage in fishes separates very early from the pharyngeal floor, even before all yolk has disappeared. The clump of cells grows as it moves to its final location, with part of this movement only apparent, being accounted for by rearrangement of cervical tissues. Hoar (1939) describes a solid anlage in the Atlantic salmon (Salmo salar). However, this is an exception, and Vivien (1958) in his review characterizes the anlage of teleosts as being typically vesicular.

Formation of the thyroid involves two stages: proliferation of cells, and formation and growth of follicles. Follicle formation takes place in two ways. First, the primary follicles are formed by separation of groups of epithelial cells from the anlage. These balls of cells then fill with secreted colloid. This colloid is at first chromophobic, then later chromophilic (Hoar, 1939). Some colloid may accumulate before
detachment from the anlage (Hill, 1935). Next, secondary follicles are formed from primary follicles by formation of solid or hollow buds, or by constriction of follicles. Hoar found that colloid filled follicles were present in Salmo salar at hatching. Gudernatsch (1911), after studying the thyroid glands of twenty-nine species of fish from several orders, made the observation that colloid is found earlier in the more primitive orders.

It is interesting to note the difference between teleost thyroid embryogenesis and that of the lamprey subpharyngeal gland. In the latter, the thyroid anlage is a long groove between the second and fifth visceral arches. A tubular structure is formed by overgrowth of the groove from the sides. In this way a duct is formed, the opening of which is at the level of the fifth visceral arch. At metamorphosis, portions of the subpharyngeal gland are transformed into a ductless thyroid gland consisting of scattered follicles (Gorbman and Berman, 1962).

3. Anatomy

a. General remarks

A few species of teleosts have a compact gland, others in addition possess a loose capsule, but typically the gland consists of separate follicles scattered along the ventral aorta (Hoar, 1939). A compact gland has been described for Sarda sarda (Gudernatsch, 1911) for Thunnus thynnus, Istriophorus orientalis, Seriola aureovittata, and Seriola quinqueradiata (Honna, 1956 a,b,c) and for Salaria (Harms, cited by Vivian, 1958). Loosely encapsulated glands have been found in
Xiphias gladius (Addison and Richter, 1932); Coleoichtha felis (Fowler, 1932); Gymnarchus niloticus (Thompson, 1950); and in the family Sparidae (Matthews, 1948). By use of thyroxine, Harms (op. cit.) obtained a change in the thyroid gland of Periophthalmus from scattered follicles to a large, compact gland with a surrounding membrane. Vivien and Geiser (1932) got similar results with thiourea in Lebistes reticulatus. In some of the foregoing species (e.g., Thunnus thynnus), the gland comprises two separate lobes, anterior and posterior.

The following statements will be mainly concerned with the unencapsulated type of gland, inasmuch as this is the type found in the two species of suckers used in this study. A particularly pertinent study is that of Hill (1935) on a holostean fish, Amia calva. This fish also has a diffuse, unencapsulated gland. Additional descriptions of anatomy and histology of the teleost thyroid are found in the experimental literature as well. These include a large number of studies on salmonids in relation to the parr-smolt transformation. The literature on this phenomenon is reviewed amply by Baggeman (1960). Because the metamorphic changes involved are more physiological than morphological, the salmonids are mentioned here only in part.

b. Location of the gland

Considerable variation exists between species and within species in the relation of the gland to the ventral aorta. In some it is located above the aorta, while in others it is located below
(Gudernatsch, 1911). Few follicles, except in the case of heterotopic thyroids, are found posterior to the base of the third aortic arch. Many of the follicles closely adhere to the ventral aorta, although nutrition is not derived from this vessel which contains venous blood (Gudernatsch, 1911; Hoar, 1939). Although the location of the gland is often described as being at the bifurcation of the ventral aorta, much variability occurs between species in the location of the bifurcation (Silvester, 1925). In some species, the anterior aortic arches spring from the two branches; whereas in others, such as the tilefish, Lophiophilus, all four of the aortic arches spring from the single ventral aorta. Therefore, the gland is better described as lying in the area of the bases of the first and second aortic arches in the majority of species.

Although the thyroid gland in fishes has been described even recently as being paired (Weichert, 1958), most workers have concluded that the gland is unpaired (Gudernatsch, 1911; Hoar, 1939). The separate anterior and posterior lobes found in certain species (e.g., Thunnus thynnus) do not correspond to the paired condition in the amphibians.

e. Heterotopic thyroid tissue

Some recent studies concerned with the subject of abnormally located (heterotopic) thyroid tissue in fishes are reviewed by Baker-Cohen (1959). The lack of a capsule is thought to allow migration of the follicles into the branchial arches and other structures (Baker-Cohen, 1959; Barrington, 1963). Thus, although the follicles are
found near the bases of the first and second aortic arches in young fish, the definitive location may be much more extensive. Gudernatsch (1911) observed this tendency of follicles to migrate, and stated that the main direction of this migration was toward the heart rather than anteriorly. Several cases have been reported of thyroid follicles in areas quite distant from the original location. Normal occurrence of heterotopic thyroid follicles is reported in several species, including the goldfish, Carassius auratus, (Baker-Cohen, 1959). Follicles may be found in the heart, head, kidney, eye, spleen, liver, base of gills, connective tissue near the ear, cranial bone surface, pseudobranch, air bladder gland, and the intestinal wall. In order to locate the heterotopic tissue, radioiodine autoradiography is used. In the platyfish (Xiphophorus), heterotopic thyroid tissue does not develop until at least two months after birth, although the thyroid is fully developed before birth. Hill (1935) proposes that the blood vessels actually pull the follicles along with them during early growth. Baker-Cohen (1959), however, suggests that the thyroid displacement is an active migration of thyroid cells or follicles from the pharyngeal area along the outer walls of blood vessels. This latter theory coincides with the mode of migration of thyroid follicles mentioned by Gudernatsch (1911).

Radial dispersion of follicles is largely dependent upon the overall shape of the subpharyngeal region. The lateral extension generally exceeds dorsoventral extension, particularly in those species with a broad subpharyngeal region. In nearly all of the species studied there is a decrease in mass of the gland posteriorly (Gudernatsch, 1911).
a. Connective tissue

The area in which the thyroid follicles lie is described by Gudernatsch (1911) as consisting of wide meshed connective and fatty tissue. Gortman and Bern (1962) classify it as loose connective tissue which may contain muscle fibers. Barrington (1963) remarks that fibrous elements are present. Gudernatsch (1911) found that Salvelinus fontinalis and Sarda sarda possess smooth muscle fibers suspended in the connective tissue. Moor (1939) describes the area as a "delicate fibrous network containing numerous clear spaces representing fat vacuoles of adipose cells." Addison and Richter (1932) report scattered elastic fibers and small fat cells in the connective tissue around the compact gland of Xiphias gladius.

e. Blood supply

Although the follicles are closely associated with the ventral aorta, this vessel probably does not service the gland. A thyroid artery has been reported by Silvester (1905) in several species of fish. It arises as a dorsal branch of the united right and left fourth commissural arteries, which in turn originate from the second afferent branchial arteries (Fig. 8). Moor (1939) was unable to find this artery in Salmo salar. Hill also states that the common ventral opercular artery and the coronary artery pass through the thyroid region without giving off branches in Amia calva. Shearer (1930) finds no artery other than these two passing to and through the thyroid region. The most anterior portion of the thyroid may receive its blood supply from a pair of thyro-spiracular arteries which arise from the first pair of
afferent branchial arteries (Vivien, 1958).

Some differences exist in the literature as to the venous blood source. According to Vivien (1958) and Hill (1935), the principal vein draining the thyroid area is the inferior jugular vein. Hill states that it breaks up into several anastomosing branches around the follicles. In contrast, the principal vein, according to Oudematsch (1911) is a thyroid vein. It also drains the muscles ventral to the aorta, then leads directly into the sinus venosus. The capillaries investing the follicles drain directly into this vein. Hoar (1939) was unable to find a thyroid vein in *Salmo salar*, and states that lymph, not blood, plays the dominant role in vascularization of the gland. He found a large lymphatic sinus along the ventral aorta in the thyroid region. Burne (1926) also describes an extensive lymph sinus in this area which is continuous cranially with the inferior jugular vein. Florentin's (1927) claim that the gland is vascularized only by blood capillaries is doubted by Hoar (1939). Vivien (1958) states that a large lymphatic sinus in the subpharyngeal region near the thyroid is a general characteristic of many teleosts. Hoar reports that the follicles in *Salmo salar* are often found to be almost floating in a sea of blood or lymph.

f. Nerves

According to Barrington (1963), non-medullated nerve fibers are present in the thyroid region, but these are all vasomotor. He states that it is generally agreed that the secretory epithelium lacks any motor innervation. Descriptions of nerves in the thyroid area are
missing from most anatomical studies of the teleost thyroid gland.

4. Histology

The histology of the teleost thyroid gland is similar to that of other vertebrates. Secreted colloid is stored in hollow follicles whose walls are made up of secretory epithelium (Barrington, 1963). The epithelial cells may vary from flat, almost squamous to tall columnar cells (Gortman and Bern, 1962).

Two types of cells are reported in the thyroid gland: the normal secretory cells (chief cells), and the degenerative Langendorf "colloid cells" (Bloem and Fawcett, 1962). The latter are reported by Hoar (1939) in older individuals, but rarely in part or small stages of Salmo salar.

The only form of a basement membrane apparent in Salmo salar is a delicate layer of connective tissue (Hoar, 1939). This, however, occurs only occasionally. He suggests that the epithelial cells rest directly on the endothelium of the blood vessels in some cases. Some follicles have certain portions of their perimeters with only a thin membrane between the colloid and the endothelium of the blood or lymph vessel. Other workers concur with these findings. Rhodin (1963) reports a very thin basement membrane in his electron photomicrographs of the thyroid.

Cell boundaries appear distinct in active glands with columnar cells, but indistinct in non-active glands with low epithelium (Vivien, 1958). In the cod and salmon, follicles which are apparently inactive may be replaced by cords of epithelial cells called "cordons de Wolfer"
Nuclei are very large, especially in early stages. With an increase in age, the amount of cytoplasm in relation to nuclear size increases in *Salmo salar* from practically none to several times the nuclear volume (Hoar, 1939).

Cell fragments have been reported in the colloid (Buchmann, 1940), a fact which has led to speculation that secretion in the thyroid gland may be occasionally holocrine in addition to the usual merocrine secretion. Corbman and Bern (1962) state that disintegrated epithelium may be found in the colloid of all vertebrates, but most commonly in teleosts and cyclostomes. Pickford (1957) mentions two methods of colloid release: the usual method of basal secretion, and disruption of the follicle wall.

Thyrotropic hormone induces the following morphological changes in order: (1) increase in cell size and nuclear size with cytological evidence of increased cell function; (2) increased vascularization; (3) growth of old follicles and proliferation of new ones (Pickford, 1957). A list of histological criteria of activity includes epithelial height, colloid condition, position and size of nuclei, degree of visibility of cell boundaries, number of secretory vacuoles in the cell, the number of mitochondria, and the size of the Golgi apparatus (Pickford, 1957; Vivien, 1958; Corbman and Bern, 1962). The last four characteristics parallel the degree of activity. Tall columnar cells indicate an active gland, while low cuboidal or squamous cells indicate an inactive gland. A colloid which is non-uniform, possibly basophilic, and in possession of chromophobic "vacuoles" or "droplets" generally is
found in an active gland. Conversely, an inactive gland has densely staining, eosinophilic colloid and few "vacuoles" or "droplets". Nuclei are basal in an active gland, whereas they are less polarized and somewhat apical in a non-active gland. Plasma membranes are less visible in non-active follicles.

Activity of the follicles varies within a single fish according to Pickford (1957). However, in fish which have been hypophysectomized for a long time, activity becomes quite uniform throughout.

Electron microscopy has revealed the following features of vertebrate thyroids. The cytoplasm contains an unusual variety of granules. The basal surface is highly folded, with the folds extending well into the cytoplasm. The apical surface possesses numerous microvilli which increase in number if the thyroid is stimulated by TSH (Gorbman and Born, 1962).

Cell height measurement has been used by several workers as an estimate of thyroid activity (Uhlenhuth, et al, 1945 a,b; Lever, 1948; Stolk, 1951 a,b; Pickford, 1953 a,b, 1954 a,b; Fortune, 1955). A high ratio of epithelial cell height to total follicle diameter, or of epithelial cell volume to total follicle volume, indicates high thyroid activity. Fortune (1955) states that the relative height of the epithelium is a measure of the TSH:TH ratio.

Matty (1960) and Swift (1960) caution against the use of only histological methods for assessing activity of the gland. They suggest the use of one other method concurrently, because epithelial heights do not always parallel production of radiothyroxine or loss of I\textsuperscript{131}. Other
methods include measuring the uptake or loss of $^{131}\text{I}$, change of radio-
thyroxine, or measurement of protein bound iodine.

Several species of fish have been reported as having an in-
creased activity of the thyroid at the time of metamorphosis, as de-
termined by epithelial height and other histological criteria. These
include *Clupea harengus* (Buchmann, 1940), the flatfishes (Sklower, 1936;
Hoar, 1951), the eel (Murr and Sklower, 1928; Hagen, 1936; Callamand
and Fontaine, 1942), *Sardina pilchardus* (Buser-Lehaye and Ruivo, 1954),
the bonefish (Basquin, 1955), and salmonids (Hoar, 1939; Baggerman,
1960; etc.). In *Sardina pilchardus*, an increase in the ratio of the
volume of the gland to the volume of the fish at metamorphosis has been
reported.

5. Function

Vertebrate thyroid glands perform various functions, and in this
respect the thyroid gland of the teleost is no exception. The basic
thyroid function in homiotherms of regulating metabolism is well known.
Between them and poikilotherms, however, there seems to be a fundamental
difference in the reaction of target organs to the thyroid hormone
(Hoar, 1957). Although there is some evidence for a calorigenic effect
in fishes, much evidence to the contrary also exists. Recently,
Barrington (1963) has stated, "In any case, it may be that the thyroid
hormones do influence the oxygen consumption of fish, but only when in-
ternal or external factors are making increased metabolic demands upon
the animals." He has observed that many workers prefer to judge the
effects of thyroxin upon amphibian metamorphosis as being metamorphic
rather than calorigenic. In fishes, likewise, the calorigenic effect seems to be less important than in warm blooded animals, although oxygen consumption has been claimed to increase in metamorphosing eels, and in salmon smolts (Barrington, 1961).

Little is known about the precise function of the thyroid gland in teleosts, but several possibilities have emerged from the available evidence. These include effects of growth, bone differentiation, pigmentation, epidermal thickening, reproduction, fin regeneration, nervous system sensitivity, osmoregulation, and nitrogen, carbohydrate, and lipid metabolism. The metamorphic function, as stated before, is uncertain (Boar, 1957; Pickford, 1957; Berg, et al., 1959; Cortman and Bern, 1962; Barrington, 1963; Cortman, 1963 b).

One must be cautious in attributing a metamorphic function to the thyroid gland just because the gland appears to be active during this period. The relationship might be coincidental, not causal (Cortman and Bern, 1962).

Iodine metabolism in most teleosts occurs in the same manner as in higher vertebrates. Two exceptions have been found, the goldfish and the sunfish (Lepomis gibbosus), in which little or no thyroxine is produced, or if produced, is done so only very slowly. This is also the case in two amphibian genera and in turtles. This is taken as evidence for questioning whether thyroxin has a normal physiological role in these animals (Cortman and Bern, 1962). Iodine uptake rates are slower for fishes, presumably because of a slower iodine excretion rate and storage of iodine in the tissues. However, iodine given to
fishes will correct thyroid tumors (Marine, 1914; La Roche, 1953b). It will also cause an increase in the activity of the gland (Vivien, 1958).

All the thyroid hormones found in higher vertebrates have also been found in some fishes, including *Umbra limi* (Gortman and Bern, 1962) and *Periophthalmus koelreuteri* (Leloup, 1956). The finding of triiodothyronine in the latter species was the first demonstration of this compound in fishes (Pickford, 1957).

Several workers have shown that teleost thyroid preparations will initiate amphibian metamorphosis (Sembrat, 1927a,b, 1954; Gunthorpe, 1932; Matthews and Ash, 1951; and Eliakova, 1954). Also, these preparations will affect the respiration rate of white rats (Smith and Brown, 1952; Kenner, et al, 1964).

But what effect do thyroid hormones have upon the metamorphosis of teleosts? Harms (1929) claims that thyroid feeding accelerated the metamorphosis of larval *Periophthalmus argentilineatus* from the usual time of 5-6 weeks to only 8 days. In other studies with several species of amphibious blennies (1929, 1935), he claims to have induced changes more suitable for terrestrial life, interpreting these changes as a second metamorphosis. Pickford (1957) suggests that many of these changes were the result of hyperthyroidism, for these fish are known to possess unusually active thyroid glands in comparison to other fishes. Gortman and Bern (1962) remark that it has been impossible for other workers to substantiate this claim of Harms.

Studies on the leptocephalus larva of the eel (*Anguilla*) shed further light on this question. Sklower (1930) claims a metamorphic
role for the thyroid on the basis of simultaneous increased activity. Hagen (1936), however, finds that actual colloid resorption began only at the end of metamorphosis. Also, in spite of an increase in the size of the gland and the number of follicles, Francois (1941) claims that colloid release was associated with resumption of feeding.

Wilter (1944 a,b) kept larval eels in the laboratory without feeding and noticed that metamorphosis was completed without any increase of thyroid activity. He performed more experiments later (1946) using thyroxine in increasing concentrations on the transparent glass eel, and was able to accelerate metamorphosis. Constant thyroxine levels had very little effect, and decreasing concentrations retarded growth of the stomach. He cautions that the effects obtained were gradual, however, and may not be attributable to the thyroxine.

The parr-smolt transformation of salmonids has been studied by a number of workers. Some studies show that thyroxine is capable of producing changes resembling the natural phenomenon (Landgrebe, 1941; Robertson, 1949; La Roche, et al, 1950; La Roche and Leblond, 1952; La Roche, 1953 a). However, the transformation lacks some of the changes which occur in natural smoltification (Fontaine and Baraduc, 1954). Baggerman (1960) and Barrington (1961) review the problem in this group of fishes. Inasmuch as this problem may be one of physiological metamorphosis more than morphological, less attention will be paid to it here, although this is not to say that it is not an important part of the metamorphic phenomenon. The reader is referred to Hoar (1953, 1957, 1958), Fontaine (1954), Fontaine and Leloup (1962), Barrington (1963), and Baggerman (1959, 1962, 1963) for
further information. The thyroid gland of diadromous fishes such as salmonids and eels has been linked to an osmoregulatory and migratory role by many workers (Baggerman, 1960; Barrington, 1961; etc.). Inasmuch as metamorphosis occurs at the same time as migration, the picture is still not clear insofar as thyroid function in metamorphosis is concerned.

Although antithyroid drugs have been used extensively in relation to other functions of the thyroid gland, little has appeared in the literature concerning their use in attempting to inhibit teleost metamorphosis. Some work on acipenserines will be mentioned later. The general effect of these drugs is to prevent normal morphological and physiological processes. In the acipenserines, development was retarded (Iakovleva, 1949). The salinity preference of juvenile Pacific salmon during the parr-smolt transformation was altered (Baggerman, 1963). Both of these cases involved decreased thyroid activity.

The compact thyroid glands of some teleosts may be removed by surgical methods (Matty, 1957), but in fishes with diffuse glands this is not possible. However, radiiodine given in large overdoses results in an effective thyroidectomy. This procedure has been used by several workers (La Rocha and Leblond, 1954; Fromm and Reineke, 1956; Oliverseau, 1957), but not in connection with metamorphosis.

Thyrotropin has been demonstrated to be present in teleosts (Fontaine and Fontaine, 1962; Barrington, 1963). The goldfish thyroid responds to TSH from various vertebrate donors (Gorbman, 1940,
1946), and hypophysectomized male Fundulus are very sensitive to mam-
malian TSH. Reciprocal tests, however, show that although pituitary
extracts from a pleuronectid fish evoke a position response in the
thyroids of goldfish and a salamander (Petrachonus attenuatus), they
produce no response on the thyroids of guinea pigs and a lizard
(Sceloraurus occidentalis). This has been taken to indicate some
measure of class specificity (Pickford, 1957).

A number of studies show that the teleost adenohypophysis
does not become fully differentiated until after the thyroid has
become functional. In the bonefish (Albula vulpes), Rasquin (1955)
finds that the basophils cannot be identified positively until the
end of metamorphosis, while acidophils and chromophobes are readily
identifiable earlier. Yet, the thyroid of this fish is active despite
the immaturity of the basophils presumed to be responsible for the
production of TSH. Similar results have been reported in other
species, including the eel (Hagen, 1936), Abramis brama (Irikhimovich,
1958), and Clupea harengus (Buchmann, 1940). But, of course, the
undifferentiated hypophysis of amphibian larvae is capable of stimu-
lating the thyroid if the glands are placed closely enough together
(Etkin and Ruth, 1939). On the other hand, Pickford (1957) mentions
that in acipenserine fishes the hypophysis plays no part in the
regulation of thyroid function until well after metamorphosis. In
reference to a study by Evropeitseva (1949) on the whitefish,
Coregonus lavaretus ludoja, she remarks, "It is not clear, however,
that the increase in cell height does not reflect the beginning of
thyrotropic regulation, despite the relatively undifferentiated state of the pituitary."

The Thyroid Gland of Other Poikilotherms

Comparative aspects of thyroid gland anatomy and function are covered in a number of reviews (Goldsmith, 1949; Lynn and Wachowski, 1951; Pickford, 1957; Gorbman and Bern, 1962; Barrington, 1963; Gorbman, 1963 b).

1. Agnatha

Marked metamorphosis occurs in the lamprey. Thyroid activity is present in certain portions of the subpharyngeal gland, but extracts of the gland are not capable of inducing amphibian metamorphosis until after metamorphosis of the ammocoetes larva into the adult lamprey (Barrington, 1959, 1960). All attempts to induce precocious metamorphosis in lamprey larvae with thyroxine have failed (Lynn and Wachowski, 1951).

No metamorphosis occurs in myxines, and the thyroid gland consists of scattered, unencapsulated follicles (Gorbman, 1963 a), as does that of the postmetamorphic lamprey.

2. Elasmobranchii

The compact thyroid gland of elasmobranchs is encapsulated and can be removed surgically. Iodine metabolism resembles that of higher vertebrates, but with lower functional level. Elasmobranch thyroid preparations will initiate amuran metamorphosis. In addition to making the preceding statements, Pickford (1957) suggests that the adult
pituitary contains little or no thyrotropin.

3. Chondrostei

Considerable work has been done on this group of fishes by several Russians. Pickford (1957) reviews the literature. *Polypterus senegalus* possesses a delicate capsule around two separate masses (Thomopoulos, 1951). However, the thyroid gland of acipenserines resembles that of most teleosts in consisting of unencapsulated follicles along the ventral aorta. The pituitary is also similar to that of teleosts though somewhat more primitive. The thyroid-pituitary axis functions similarly in acipenserines and teleosts.

Metamorphosis occurs in several species of sturgeon. Certain studies correlate metamorphosis in *Acipenser stellatus* with increased activity of the thyroid (Olifan, 1945). After using thiourea, a thyroid inhibitor, on the same species during the early stages, Iakovleva (1949) concludes that no thyrotropin is secreted during post-embryonic, pre-larval, or larval periods. The treatment did, however, result in a definite retardation of development, thus indicating that the thyroid plays a morphogenic role in larval stages, but may be independent of pituitary control. Other workers have been able to accelerate early morphogenesis by treatment with thyroxine (Gerbil'skii and Zaks, 1947). Their experiments show that thiourea decreases oxygen consumption during the larval period, but that thyroxine restores it to normal. Pickford (1957) concludes that, "The acipenserine thyroid plays both a morphogenic and a metabolic role during post-embryonic development, at a time when the gland is functionally independent of
pituitary control, but that thyrotropic stimulation comes into play at the time of seaward migration." Thyrotropin is present in the adult pituitary.

4. Amphibia

The evidence for direct involvement of the thyroid in amphibian metamorphosis is almost unequivocal. Etkin (1955, 1964), Frieden (1961), and Kollros (1961) review this subject quite thoroughly. Two statements by Etkin (1964) sum up the fundamental knowledge uncovered during the first half of this century: "(1) Metamorphic change in the tissues is activated primarily by the hormone or hormones produced by the thyroid gland. (2) The metamorphic activity of the thyroid gland is controlled by a specific thyroid stimulating hormone (TSH or thyrotropin) secreted by the anterior lobe of the pituitary." Studies by Etkin (1930) show a parallel between the activity of the gland and its histology. In 1963 he proposed that the thyroid and pituitary work together to produce a self-accelerating and retarding system for production of increasing amounts of thyroxine up to metamorphic climax, followed by decreasing amounts after climax.

The thyroid gland was first linked to amphibian metamorphosis by Gudernatsch (1912) discovery that extracts of thyroid glands were able to induce precocious metamorphosis. Removal of the thyroid prevents metamorphosis (Allen, 1916; Hoskins and Hoskins, 1917). Re-implantation of even undifferentiated thyroids compensated completely for failure to metamorphose in thyroidectomized animals. Extracts from premetamorphic thyroid and pituitary glands do not induce pre-
cocuous metamorphosis, whereas extracts from metamorphic glands do (Swingle, 1923; Slowikowska, 1923; Allen, 1931). Removal of the pituitary gland inhibits metamorphosis (Adler, 1914; Allen, 1916; Smith, 1916), and results in the thyroid remaining quite small (Smith, 1920). Reactivation of the thyroid occurs upon reimplantation of the hypophysis, even at sites far from the normal location, if the animal has not been thyroidectomized (Allen, 1927; Grant, 1931). Thyroid inhibitors prevent metamorphosis when given to tadpoles (Gordon, et al, 1943; Lynn, 1948). The volume of the gland in proportion to body volume increases at metamorphosis, and afterward decreases. The gut is also affected by thyroxine (Etkin, 1964).

Other Factors in Metamorphosis

Some workers suggest possible involvement of the adrenal cortex in metamorphosis (see Barrington, 1961). Etkin (1964) states that the evidence conflicts, and suggests that metamorphosis of amphibians most probably results from operation of the thyroid gland only. This may or may not be true of fishes. Sterba (1955) claims inducement of partial metamorphosis in lampreys with corticotropin.

Factors Affecting Thyroid Gland Activity in Poikilotherma

Although other hormones may affect the function of the thyroid gland, Etkin (1964) argues against "complex interactions between many hormones for an understanding of the patterning of metamorphic events" in amphibians. He states, "We must conclude ... that whatever pharmacological interactions may occur, no physiological role in the normal metamorphic process has been demonstrated for any of the steroid hormones."
The metamorphic process in fishes may be somewhat different. The possibility exists of a synergistic action between growth hormone and thyroxine in the early stages of fish development. According to Goerksen and Bern (1962), the less spectacular morphologic effects of thyroxine (other than thyroid-controlled metamorphosis in amphibians) may be considered maturational.

The thyroid gland exhibits a seasonal rhythm. The effects of reproduction, photoperiod, temperature, and iodine content of the water are discussed by Raggeman (1960), Swift (1960), Matty (1960), Hickman (1962), and Barrington (1963). Although all these have been shown to affect thyroid activity, the problem is a complex one involving interaction of several factors, and has not been sufficiently worked out to allow an exact delineation of the separate parameters. In contrast to homiotherms, the peak of thyroid activity in poikilothersms usually occurs during the spring and early summer. Variation of the cycle occurs between species. Seasonal fluctuations in the iodine content of a salt water fish (Flatichthys stellatus) and a fresh water fish (Coregonus clupeaformis) have been studied by Hickman (1962). In the latter fish, there was a much greater seasonal stability than in the former where iodine in the blood serum and in the thyroid gland varied seasonally in a manner parallel to the large quantitative changes in the environment. Seasonal changes were obvious in both species, but peak activity occurred at different times for each, in summer for P. stellatus and in late fall for C. clupeaformis. It is important to note Hickman's assertion that these changes were not associated with any one factor in the physical environment.
Diet may also affect thyroid activity (Marine, 1914; Hoar, 1951, 1959). The change of diet occurring in some metamorphosing fish could conceivably cause a change in thyroid activity (Francois, 1941; Darrington, 1960).
MATERIALS AND METHODS

Periodic collections of newly hatched suckers were made with a dip net during June, July, August, and early September from an oxbow slough of the Bitterroot River about two miles upstream from Ft. Missoula. After fixation in 10% buffered neutral formalin, the fish were transferred to 7% buffered neutral formalin for preservation. The collections included two species of suckers, \textit{Catostomus catostomus} and \textit{Catostomus macrocheilus}. No attempt was made to separate the two species because they are so similar in their prolarval and early postlarval stages. Late postlarval stages were readily identified by fin ray counts. Sample groups from these collections have an approximately equal number of each species. During collections, observations on fish behavior were noted, as well as pertinent information on the slough.

Certain morphological traits were found suitable for characterizing six arbitrary stages encompassing the metamorphic period. Descriptions of the stages chosen are as follows:

\textbf{Stage one (prolarval):} some yolk remains in most; caudal fin rounded, with about 15 principal rays; mouth terminal with no sign of a protracatile apparatus; notochord partially upturned; swim bladder with a small anterior bulge; finfold elevated at the site of the future dorsal and anal fins, but no rays present; gills partially exposed (Fig. 1). Total lengths range from 12.0-14.0 mm (Table 2).

\textbf{Stage two (prometamorphic):} pelvic rudiments appear; oper-
Column covers gills completely; swim bladder subdivided; caudal fin slightly margined with 19 principal rays (Fig. 1). Total lengths range from 15.0-16.5 mm (Table 2).

Stage three (early metamorphic climax): pelvic fins with obvious rays, but fins do not extend beyond the ventral edge of the ventral finfold; dorsal finfold disappears (Fig. 1). Total lengths range from 16.5-18.5 mm (Table 2).

Stage four (metamorphic climax): pelvic fins definitely extend beyond ventral edge of ventral finfold; ventral finfold somewhat reduced anterior to the pelvic fins; first loop of intestine begins to form (Fig. 1). Total lengths range from 17.5-20.5 mm (Table 2).

Stage five (postmetamorphic): finfold almost gone; scales begin to develop; intestine has two full 180° turns; nostril divided or almost divided; mouth ventral with obvious protractile apparatus (Fig. 1). Total lengths range from 21.5-23.0 mm (Table 2).

Stage six (postmetamorphic): intestine with a posterior turn in the anterior loop; nostril definitely divided, with the two openings spaced widely apart (Fig. 1). Total lengths range from 27.0-36.5 mm (Table 2).

The average total lengths for each stage are graphed (Fig. 2); and the coiling of the gut, migration of the mouth, and dividing of the nostril are diagrammed (Figs. 3, 4, 5, 6). The average total length of each stage, together with a comparison of different nomenclatures of these immature fish appears in Table 1.

For each stage the formalin fixed fish were treated as follows:
Two were treated in toto by clearing in 2% KOH, staining with Alizarine Red S, and preserving in glycerine (Evans, 1948). Three were dehydrated in alcohol and cleared in methyl salicylate. Six were imbedded in paraffin, serially sectioned at 10 microns, and stained by the Periodic Acid-Schiff technique (Kasten and Burton, 1959).

Several adult fish were collected. The heads were removed and fixed in Carnoy's solution, then stained with an alcoholic silver solution (Rosenthal and Wingstrand, 1961). The individual thyroid follicles thus appear light brown against a cream-colored background. Because of the diffuse nature of the gland, study of the thyroid is greatly facilitated by this method. These fish were grossly dissected for examination of the thyroid, and of follicle migration.

To estimate the activity of the gland at the different stages, the total number of follicles per fish were counted in the PAS stained material. Also, each follicle was measured for average epithelial cell height, and for greatest and smallest follicle diameter. The average of the last two measurements was used to calculate the ratio of the epithelial height to the average follicle diameter. Hereafter, this ratio shall be referred to as the ER/FD ratio. Inasmuch as individual follicles exhibit variable epithelial height, it was necessary to visually select a typical cell height for measurement. In addition, a number of nuclei were selected at random for measurement of their length. Some previously used methods for histological estimation of activity, such as Fortune's (1955) method of paper cutouts, were not used because of the relatively large number of epithelial cells which were in solid clumps with little or no colloid.
Prudence must be used in evaluating thyroid activity by only histological criteria. The present method requires special caution because of the variability of epithelial height. Possible errors are considered in the discussion.
TABLE 1. COMPARISON OF STAGES

<table>
<thead>
<tr>
<th>Stages of, This Study</th>
<th>Total Length (Ave.) in mm.</th>
<th>Stage of Metamorphosis</th>
<th>Stages of Young Fish</th>
<th>Stage Designation Number of Winn and Miller (1954)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.9</td>
<td>Prometamorphosis</td>
<td>Prolarvae</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>15.8</td>
<td></td>
<td></td>
<td>37,38</td>
</tr>
<tr>
<td>3</td>
<td>17.3</td>
<td>Metamorphic Climax</td>
<td>Postlarvae</td>
<td>39,40</td>
</tr>
<tr>
<td>4</td>
<td>18.9</td>
<td></td>
<td></td>
<td>41</td>
</tr>
<tr>
<td>5</td>
<td>22.1</td>
<td>Postmetamorphosis</td>
<td></td>
<td>42</td>
</tr>
<tr>
<td>6</td>
<td>30.9</td>
<td></td>
<td>Juveniles</td>
<td>--</td>
</tr>
</tbody>
</table>

* Studies on several other catostomid species
Figure 1. Photographs of the six stages of larval suckers.
Figure 2. Graph of the total length of fish (Ave. for each stage)
Figure 3. Diagram of coiling and elongation of the gut.
Figure 4. Diagram of mouth migration (external view).
Figure 5. Diagram of mouth migration (mid-sagittal section).

Figure 6. Diagram of nostril division (in relation to eye).
ANATOMY

In all larval stages, the thyroid gland of the two catostomids is located along the ventral aorta at the bases of the first two aortic arches (Figs. 7, 8). It is unencapsulated and consists of separate, scattered follicles occupying the loose, mesenchymatous, fatty connective tissue which comprises a large part of the hypoglossal region.

The limits of the gland are, roughly, the hyoid bones anteriorly, the sternohyoideus muscle ventrally, and the basibranchial bones and attached muscles dorsally. The posterior limit is rather indefinite. Although the gland rarely extends to the third aortic arch, the only apparent morphological barrier to further posterior movement of the follicles appears to be the narrowing of the region of connective tissue above the ventral aorta posterior to the first aortic arch (Fig. 7). Here, there is little connective tissue because the branchial muscles and the sternohyoideus muscle are close to the ventral aorta. This is especially true at the base of each gill arch where the branchial bones and their muscles take up much of the area dorsal to the ventral aorta. Between the bases of the first and second gill arches there is a greater amount of connective tissue which allows radial spreading of the follicles, but not nearly so much as in the more anterior region between the basihyal, hypohyal, ceratohyal, and the first basibranchial bones. All these bones are still cartilaginous in the stages examined.
The general shape of the area containing the follicles is thus loosely divided into two regions, anterior and posterior. The anterior region is the broader one lying anterior to the base of the first gill arch. The posterior region is more restricted and lies posterior to the base of the first gill arch. A few follicles are present between the two regions. In both regions, follicles are almost exclusively dorsal to the ventral aorta.

In both regions the follicles are generally round. Some irregularities in shape do occur, however (Figs. 20, 23). In areas where the connective tissue is restricted, such as at the bases of the first and second gill arches, some elongated follicles are present. These are oriented longitudinally along the ventral aorta. In later stages, more irregularly shaped follicles appear in both regions (Fig. 12). Often, the sides of the follicles may be flattened, either by abutment against another follicle, or by close adherence to a blood or lymph vessel.

The anterior follicles are mostly associated with both veins and lymph spaces, while the posterior follicles are associated almost exclusively with veins. The arrangement of the blood vessels (Fig. 8) resembles that of the angler fish, Lophiatus, which Silvester (1905) described from injected specimens. However, the thyroid vein which he described could not be found.

The ventral aorta does not divide until it has given off the first aortic arch and descended through the anterior thyroid region. Although the follicles are close to the aorta, they are much more intimately associated with the inferior jugular vein and its branches,
and in the anterior region with the lymph vessels. A pair of thyro-
spiracular arteries arise from the first afferent branchial arteries.
These descend through the anterior thyroid region, giving off branches.
A few follicles are closely applied to these arteries in later stages.
The thyroid artery is an anterior branch of the posteriorly directed
hypobranchial artery. It arises from the junction of the two com-
misural arteries which branch from the second afferent branchial
arteries. The thyroid artery runs beneath the ventral aorta and
gives off small branches dorsally to the posterior thyroid region.

The main venous supply of the thyroid is the inferior jugular
vein (Figs. 8, 20). It is quite small and incompletely differentiated
until about stage five. It joins the common cardinal vein dorsal to
its junction with the sinus venosus. Anterior to this junction it
courses above the ventral aorta. The anterior thyroid region receives
many branches from the inferior jugular vein (Fig. 8), and thus forms
a plexus. The branches of this plexus become fewer in number and
larger in later stages, as if they are joining. Anterior to the
plexus, the jugular vein is broken up into two main branches from
which numerous smaller branches extend to drain the more ventral areas
of the hypoglossal region.

The presence of blood cells in the veins, arteries, and venous
sinuses distinguishes them from another series of large, irregularly
shaped vessels located in the anterior region of the thyroid and
further forward. These vessels contain no blood cells and are inti-
mately associated with a large number of thyroid follicles. In con-
trast with the veins, which have thicker walls, they have only a simple endothelium. These are very likely lymph vessels comprising a vast lymph sinus in the connective tissue anterior and posterior to the hyoid bones (Figs. 8, 22, 23). Midway in the anterior region of the thyroid there are connections between these lymph sinuses and the veins. Near the connections a few blood cells are present in the lymph spaces. In addition to the venous plexus and lymph sinuses, numerous capillaries are found in the connective tissue. Many of these run directly against the thyroid follicles.

Dark brown pigment appears around the ventral aorta in stage three. By stage six the amount has increased greatly, and considerable pigment may also be present around the inferior jugular vein and the smaller arteries.

Follicles from stage six show a more extensive distribution than those in stage one (Fig. 21). Some are at the bases of the gill arches in stage six, but still confined to the hypobranchial region proper. In adult fish, a few follicles are in the gill arches. An occasional follicle may be found ventral to the ventral aorta in later stages of larval fish.

HISTOLOGY

Follicular counts and measurements, epithelial height and nuclear length measurements, and calculated values of the EH/FD ratio are summarized in Table 2. The EH/FD ratios are related to total length of fish (Fig. 13).

Most of the histological changes of the gland are relatively
small and fairly gradual from one stage to the next. The general trend is as follows. The average diameter of the follicles increases from 15.0 microns in stage one to 38.6 microns in stage six, (Fig. 11). Likewise, the number of follicles increases from an average of 21.8 to 146.7 (Fig. 15). Irregularity of the follicles increases with age (Fig. 21). The number of involuted follicles, as well as the number of follicles which are lobed also increase with age. Numerous small follicles are present in all stages. Stratification of epithelial cells is found on parts of the perimeter of a number of follicles in all stages. Small groups of epithelial cells without colloid also are present. The diameter of the colloid increases from an average of 10.4 microns in stage one to 26.3 microns in stage six (Fig. 16). The actual height of the epithelium increases slightly, as does the length of the nuclei (Figs. 17, 18). The shape of the nuclei remains the same throughout—an oval, somewhat flattened disk. Cell outlines become increasingly more distinct, but are not well-defined even at stage six. The shape of individual epithelial cells in stage one is somewhat irregular, becoming more rectangular in cross section in later stages. This irregularity is caused by the distension of the plasma membrane by the nuclei which are relatively large compared to the amount of cytoplasm. The number of small chromophobic "granules" in the colloid increases markedly, as does the number of the much larger "secretory droplets" around the edge, although they are present even in stage one. The number of "secretory droplets" is quite variable between fishes of the same stage.

The relation of the follicles to the capillaries, veins, and
lymph vessels is similar in all stages. Commonly, the follicles appear to have no epithelial cells on sides where they contact the endothelium of vascular channels. A basement membrane is not visible beneath the epithelial cells. However, where epithelial cells seem missing between colloid and endothelium, a thin acidophilic film usually appears to separate them. Occasionally, however, this film appears to be absent, with the colloid seeming to rest directly against the endothelium of lymph vessels or capillaries.

Descriptions and measurements of the follicles for each stage are as follows:

**Stage one** (Fig. 19). The follicles are small, averaging 15.0 microns in diameter. The average number of follicles is 21.8. They are quite spherical, but may be grouped in irregular clumps, some containing several separate accumulations of colloid. The colloid, which has an average diameter of 10.4 microns, has few "secretory droplets" and "granules". The epithelial cells may be characterized as regular cuboidal, although they vary within a single follicle from low cuboidal to regular cuboidal. Their average height is 2.3 microns. The amount of cytoplasm is small, causing the nuclei to fill most of the cell and distend the plasma membrane, especially on the basal side. Nuclei average 5.2 microns in length. Plasma membranes are indistinct. The ER/FD ratio is 0.30.

**Stage two** (Fig. 22). The diameter and number of follicles have increased to average 20.5 microns and 25.8 respectively. The follicles are more separated than in stage one, and a few are irregularly shaped.
The average diameter of the colloid has increased to 15.4 microns and it contains more "secretory droplets" and "granules". The average epithelial height is 2.5 microns. The amount of cytoplasm has increased, but the nuclei, which now average 5.7 microns in length, still distend the plasma membrane. Cell outlines are slightly more distinct between adjacent epithelial cells. The ER/FD ratio is 0.25, a decrease from stage one.

**Stage three (Fig. 22).** The diameter and number of the follicles has increased. They now average 23.5 microns and 32.3. More follicles are irregular or elongated. Average colloid diameter has enlarged to 19.1 microns, while average epithelial height has lowered to 2.2 microns. Nuclear length has decreased also, to 5.4 microns, as has the ER/FD ratio to an average of 0.18.

**Stage four (Fig. 23).** The follicular diameter has diminished to an average of 23.3 microns, despite the fact that this stage contains individual follicles which are larger than those of stage three. The number of follicles, however, continues to grow and now averages 19.4. Involuting follicles are more numerous and the degree of involution is more apparent. The colloid has reduced in diameter to 18.5 microns, paralleling the decrease in follicular diameter. There is an increase of average epithelial height to 2.6 microns, of nuclear length to 5.7 microns, and of the ER/FD ratio to 0.22. The nuclei of a large number of cells are somewhat basal in position.

**Stage five (Fig. 23).** The average follicular diameter and number of follicles continues to increase. They are 28.7 microns and 66.0 re-
spectively. The average diameter of the colloid has enlarged to 23.3 microns, paralleling the resumed growth in average follicular diameter. The "secretory droplets" around the edge of the colloid are more numerous, as are the "granules" in the center of the colloid. The epithelial cells are now primarily cuboidal, although considerable variation still exists and many cells are low cuboidal. Their average height is 2.8 microns. The nuclei no longer distend the plasma membranes, although their average length has increased to 6.1 microns. The EH/FD ratio has again lowered to 0.19.

Stage six (Figs. 20, 21). There is further growth in follicular diameter to an average of 71.7 microns. The number of follicles has increased greatly to 146.7, and the average diameter of the colloid has expanded to 52.4 microns. The shape of the follicles varies considerably. In all follicles the colloid contains an abundance of "granules" and "secretory droplets". Both may be found even in the involuted follicles which appear in appreciable number. Average epithelial height remains at 2.3 microns. Cell outlines are more visible, but still quite indistinct. The average length of the nuclei, 6.1 microns, remains the same as in stage five. The EH/FD ratio has again decreased to 0.17.
Figure 7. Diagram of mid-sagittal section through head region, showing relation of thyroid follicles to other structures.
Figure 8. Diagram of the principal arteries, veins, and lymph vessels of the thyroid region. Thyrospiracular artery, TSA; commissural artery, CA; thyroid artery, TA; hypobranchial artery, HA. (The venous plexus and lymph sinuses are more extensive than shown).
Figure 9. Diagram of ventral view of major bones of thyroid region.

Figure 10. Lateral aspect of the hypobranchial apparatus of Catostomus macrocheilus. The hyoid column has been rotated upward from its natural position (cross-hatched). URH, urohyal; SBH, supplementary basihyal; BH, basihyal; LH, lower hypohyal; UH, upper hypohyal; CH, ceratohyal; EH, epihyal; IH, interhyal; PB, pharyngobranchial; EB, epibranchial; CB, ceratobranchial; HB, hypobranchial; BB, basibranchial; PH, pharyngeal bones. (after Weisel, 1960)

Figure 11. Diagram of jaw musculature of Catostomus macrocheilus (ventral view). S, Sternohyoideus; HS, hyoideus superior; HI, hyoideus inferior; G, geniohyoid; IM, intermandibularis. (after Weisel, 1960)
**TABLE 2**

**SUMMARY OF HISTOLOGICAL MEASUREMENTS**

Values listed are means, with ranges underneath in parenthesis. All values (excluding ratios) are in micrometer units. One micrometer unit equals 0.985 microns.

<table>
<thead>
<tr>
<th></th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
<th>Stage 4</th>
<th>Stage 5</th>
<th>Stage 6</th>
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<tr>
<td>Total length of fish</td>
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<td>15.8</td>
<td>17.3</td>
<td>18.9</td>
<td>22.1</td>
<td>30.9</td>
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<td>(15.0-16.5)</td>
<td>(16.5-18.5)</td>
<td>(17.5-20.5)</td>
<td>(21.5-23.0)</td>
<td>(27.0-36.5)</td>
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<tr>
<td>Ave. number of follicles</td>
<td>21.8</td>
<td>25.8</td>
<td>32.3</td>
<td>43.8</td>
<td>66.0</td>
<td>146.7</td>
</tr>
<tr>
<td>Largest single follicle (ave.}</td>
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<td>30.6</td>
<td>38.9</td>
<td>43.3</td>
<td>58.3</td>
<td>84.8</td>
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<tr>
<td>dia.)</td>
<td>(20.0-25.0)</td>
<td>(23.5-35.0)</td>
<td>(34.0-41.0)</td>
<td>(37.5-52.5)</td>
<td>(51.0-71.5)</td>
<td>(68.5-115.0)</td>
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<td>Largest single follicle (greatest dia.)</td>
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<td>38.2</td>
<td>51.2</td>
<td>58.3</td>
<td>77.2</td>
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<td>(40.0-60.0)</td>
<td>(50.0-70.0)</td>
<td>(67.0-93.0)</td>
<td>(85.0-135.0)</td>
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<td>Ave. follicle diameter</td>
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<td>21.0</td>
<td>24.1</td>
<td>23.9</td>
<td>29.4</td>
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<td></td>
<td>(13.9-18.5)</td>
<td>(18.6-22.3)</td>
<td>(22.2-27.8)</td>
<td>(21.0-28.9)</td>
<td>(25.9-34.1)</td>
<td>(26.8-40.9)</td>
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<td>Greatest dia. of a follicle</td>
<td>16.8</td>
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<td>26.8</td>
<td>28.2</td>
<td>34.2</td>
<td>39.1</td>
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<tr>
<td></td>
<td>(15.3-20.4)</td>
<td>(21.5-26.2)</td>
<td>(26.6-31.6)</td>
<td>(24.4-33.0)</td>
<td>(28.9-35.8)</td>
<td>(33.0-47.8)</td>
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<tr>
<td>Smallest dia. of a follicle</td>
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<td>17.8</td>
<td>19.5</td>
<td>19.9</td>
<td>24.3</td>
<td>26.2</td>
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<td>(11.8-16.5)</td>
<td>(15.7-18.8)</td>
<td>(17.2-24.0)</td>
<td>(17.6-24.8)</td>
<td>(22.2-26.3)</td>
<td>(21.2-34.1)</td>
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(continued on next page)
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<tr>
<th></th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
<th>Stage 4</th>
<th>Stage 5</th>
<th>Stage 6</th>
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<tr>
<td>Ratio of dia.</td>
<td>1.22</td>
<td>1.37</td>
<td>1.48</td>
<td>1.42</td>
<td>1.41</td>
<td>1.53</td>
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<tr>
<td>(greatest/smallest)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ave. dia. of the colloid</td>
<td>10.7</td>
<td>15.8</td>
<td>19.6</td>
<td>19.0</td>
<td>23.9</td>
<td>27.0</td>
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<tr>
<td></td>
<td>(9.6-12.6)</td>
<td>(13.1-17.5)</td>
<td>(18.0-22.5)</td>
<td>(16.0-22.4)</td>
<td>(20.9-27.2)</td>
<td>(22.0-35.5)</td>
</tr>
<tr>
<td>Ave. actual epith. ht.</td>
<td>2.3</td>
<td>2.6</td>
<td>2.2</td>
<td>2.7</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>(1.9-3.0)</td>
<td>(2.4-2.9)</td>
<td>(2.0-2.6)</td>
<td>(2.3-3.3)</td>
<td>(2.5-3.5)</td>
<td>(2.4-3.3)</td>
</tr>
<tr>
<td>Ave. length of nucleus</td>
<td>5.3</td>
<td>5.9</td>
<td>5.5</td>
<td>5.8</td>
<td>6.2</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>(4.6-6.1)</td>
<td>(4.8-6.8)</td>
<td>(4.9-6.4)</td>
<td>(5.1-6.7)</td>
<td>(5.3-6.9)</td>
<td>(5.6-6.7)</td>
</tr>
<tr>
<td>EH/FD ratio</td>
<td>0.30</td>
<td>0.25</td>
<td>0.18</td>
<td>0.22</td>
<td>0.19</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>(0.27-0.33)</td>
<td>(0.22-0.31)</td>
<td>(0.17-0.19)</td>
<td>(0.20-0.27)</td>
<td>(0.17-0.21)</td>
<td>(0.13-0.21)</td>
</tr>
</tbody>
</table>
Figure 12. Graph of the degree of elongation of follicles in each stage.
Figure 13. Scattergraph of the relationship of the EH/FD ratio to the total length of fish.
Figure 14. Scattergraph of the relationship of the average diameter of the follicles to the total length of the fish. (One micrometer unit equals 0.985 microns.)
Figure 15. Graph of the average number of follicles per stage.

Total length of fish in mm.
(Ave. per stage)
Figure 16. Scattergraph of the relationship of the average diameter of the colloid to the total length of the fish. (One micrometer unit equals 0.985 microns)
Figure 17. Scattergraph of the actual epithelial height in relation to the total length of the fish.
(One micrometer unit equals 0.985 microns).
Figure 18. Scattergraph of the relationship of the nuclear length of the epithelial cells to the total length of fish. (One micrometer unit equals 0.985 microns)
Figure 19. Photomicrograph of a x-section of anterior thyroid region of a stage one fish. P, pharynx; BBC, basi-branchial cartilage; TF, thyroid follicle; VA, ventral aorta.
Figure 20. Photomicrograph of a x-section of the anterior thyroid region of a stage six fish. P, pharynx; BBC, basibranchial cartilage; VP, venous plexus; TF, thyroid follicle; TSA, thyro-spiracular artery; VA, ventral aorta.
Figure 21. Photomicrograph of a cross-section near posterior limit of the thyroid gland of a stage six fish. Just anterior to this the space occupied by the branchial muscles is occupied by connective tissue in which the thyroid follicles lie (posterior thyroid region). P, pharynx; BBC, basibranchial cartilage; BC-II, branchial cartilage II; BM, branchial muscles; TF, thyroid follicle; VA, ventral aorta; SM, sternohyoideus muscle.
Figure 22. Photomicrographs of x-section of thyroid follicles in anterior thyroid region. P, pharynx; BBC, basi-branchial cartilage; TF, thyroid follicle; LS, lymph sinus; TSA, thyro-spiracular artery; VA, ventral aorta.
Figure 23. Photomicrograph of a x-section of thyroid follicles in anterior thyroid region. P, pharynx; BBC, basi-branchial cartilage; LS, lymph sinus; V, vein; TF, thyroid follicle.
The thyroid region and the totality here are closely associated with the
thymic region, and the thymus chambers are distinctly non-existent in the posterior
totalities. The thymus chambers are distinctly non-existent in the thymic
region—except as will be indicated of association with the thyroid
region—in except as will be indicated of association with the thymic
region. In posterior cephalocaudal, the thymus appears to be entirely absent in the anterior
portion, while the thymus is present in the posterior portion of the thymus.

Proc. Nat. Acad. Sci. U.S.A. (1933) (1937) shows that the thymus is present in the man
situation different from most descriptions of the literature.

The external features in the thyroid region is similar to that at

The external features in the thyroid region is similar to that at

near the base of the first and second occipital arches.

The occipital arches are unrecognizable and scattered along the occipital area.

The occipital arches are unrecognizable and scattered along the occipital area.

The two occipital arches possess a thyroid gland which is

of primate (1934, 1937).

The determination of this term is important broad and includes a variety
be said to experience metamorphosis, so long as one keeps in mind that

With such changes in behavior and morphology, these changes may

be termed to a ventral position, and elongation of the

feeding and digestive apparatus, means, movement of the mouth from
top-water to a bottom habitat. This coalesces with the changes in

behavior of Copepodinae incertae sedis and of C. Copepoda incertae sedis from a

Discussion.

During larval transformation from larval to juvenile stages, the
inferior jugular vein and its branches. In both regions, numerous blood capillaries closely invest the follicles.

Throughout the larval period, growth and extension of the gland occurs, as indicated by the increase in number of follicles, and migration of thyroid tissue (Figs. 12, 21). The presence of numerous small follicles indicates that considerable budding takes place in all stages.

Changes in epithelial height and colloid level are often quite marked in amphibian metamorphosis. In most urodèles and some anurans, complete evacuation of the colloid occurs (Etkin, 1964). In other anurans, follicle collapse does not occur, but the changes in epithelial height and colloid level are still substantial. Metamorphic climax in Xenopus laevis, a completely aquatic animal, is accompanied by follicle collapse (Saxen, et al, 1957). Epithelial heights change over 50% and colloid levels over 500% as compared to prometamorphosis (Table 3-A). Follicle collapse does not occur in the three species of anurans studied by Etkin (1936), but the change in epithelial height is quite great (Table 3-C). Changes induced by TSH in the epithelial height of Triturus torosus (Uhlenhuth, 1945) were also very large, as well as changes in colloid levels (Table 3-B).

In comparison to studies on amphibians, the changes in the sucker during metamorphosis, other than increase in numbers of follicles, are slight. Even the increase in numbers of follicles, however, follows the growth of the sucker.

If metamorphosis in fishes is induced by the thyroid gland in a manner similar to that in amphibians, one would suspect that similar
histological changes would occur in teleosts at metamorphic climax. Such changes have been reported by some workers (see literature review), but in contrast to the amphibians it has been impossible to definitely substantiate any more than a coincidental relationship. Studies of fishes reveal that increased thyroid activity is usually accompanied by more significant histological changes than those in metamorphosing suckers. Using this source, Fortune (1955) obtained changes in ratio of epithelial volume to follicle volume of over 100% (Table 4-A). In the rainbow trout, epithelial cell height in the parr is considerably greater than in the smolt (Robertson, 1940) (Table 4-B). Photographs of the epithelium of the thyroid gland of *Abla vulnus* show a tall columnar epithelium at the beginning of metamorphosis (Pasquin, 1955).

Evidence of a marked histological crisis at the time of metamorphosis is lacking in larval suckers. The gland is not hypertrophied, nor is columnar epithelium present in any of the stages. Later stages, in fact, contain a small number of involuted follicles with flattened cuboidal epithelium. The epithelium, although variable, is typically low cuboidal to cuboidal. The changes in the EM/FD ratio are relatively small and regular, and not sufficient to prove any marked increase in hormone release during metamorphic climax (Fig. 13).

The present findings do not warrant precise interpretation. However, the growth of the gland appears to be accompanied by a gradual increase in activity of the gland. This is suggested by increases in number and size of follicles, epithelial height, nuclear length, amount
of cytoplasm, and number of "secretory droplets" and "granules" in the colloid (Pickford, 1977). The decrease in EH/FD ratio probably indicates only that the follicle is accumulating colloid. The change in this ratio is closely related to the change in colloid level (Figs. 13, 16). The change in colloid level closely follows the change in size of the follicle (Figs. 14, 15). It is likely that the decrease in the EH/FD ratio means little more than that the colloid level is increasing more rapidly than the epithelial height. The increasing number of involuted follicles reinforces this conclusion that colloid storage is taking place throughout metamorphosis. The activity, although increasing, is never great enough to evoke more release of colloid than production. This does not imply that no hormone release is taking place, and evaluation of actual hormone production and release awaits experimental verification.

The absence of a histological crisis can be interpreted from the scattergrams (Figs. 13, 14, 16, 17, 18). However, if only the averages of the six fish are consulted (Table 2, Fig. 26), one might speculate that a slight peak of activity occurs at stage four as judged by EH/FD ratio, colloid level, epithelial height, and nuclear length. The change in EH/FD ratio of similar size groups of follicles from the six stages suggests the same possibility (Table 6, Fig. 26). The present method of evaluation may not be sensitive enough to detect an extra release of hormone at this time.

The apparent decrease in activity as judged by the EH/FD ratio is partly a function of the size of the follicle (Tables 7, 3; Fig. 25). The difference between the EH/FD ratio of large and small
follicles is quite significant. Small follicles are found in higher percentages in early stages (Table 8), but this is to be expected in a gland which is producing new follicles and is in the early stages of accumulating colloid. Follicular size does not account for all the difference, however, for there is some difference in EH/FD ratio between follicles of the same size in different stages (Table 8, Fig. 26). Also, small follicles between stages differ in the relative amount of cytoplasm, indicating a fundamental difference between even newly formed follicles. Thus, the data in Table 8 and Figure 26 may more closely reflect the degree of accumulation of colloid. On the other hand, the difference in EH/FD ratio of follicles of different size in one stage may merely reflect the degree of development of the gland (i.e., the percentage of follicles which have had time to accumulate colloid). One other explanation for some of the difference may lie in an inherent error of measurement (see below). The fallibility of using the ratio of epithelial height to follicular diameter as a sole criterion of activity may be seen from the present study, particularly during periods of minor activity with colloid accumulation.

In order to judge the possible error in obtaining values for the EH/FD ratio, comparisons were made of this ratio in small and large follicles (Table 7), in anterior and posterior follicles (Table 6), and in round and oblong follicles (Table 5). A significant difference is apparent between small and large follicles, but not in the other two comparisons. Two other sources of difficulty in obtaining accurate measurements exist. One, there is considerable variation in epithelial height in one follicle. Personal judgment is necessary in selecting
an average cell height, but this bias would be similar in all cases, presumably. Too, the size of the epithelial height approaches the limit of resolution of the ocular micrometer. The smallest divisions on the micrometer equal 0.25 microns, and measurements were made to the nearest half division. The measurements, therefore, are only accurate to plus or minus 0.25 micrometer units, or approximately 0.25 microns. The error introduced here would be greater in the smaller follicles when calculating the EH/TH ratio. This may account for part of the difference between the EH/TH ratios of small and large follicles.

It may be that the thyroid gland of a few fishes functions in metamorphosis in a manner similar to that of amphibians. However, observations to date on fishes in regard to direct action of the thyroid hormone on the tissues to bring about metamorphosis are not nearly so decisive as in amphibians. Nevertheless, the thyroid, together with other factors (e.g., growth hormone), could conceivably function in metamorphosis, but in a different manner, even by a constant hormone level. This would require a differential response of the metamorphosing tissues to thyroxine, something that is known to occur in amphibians (Stahn, 1943) and possibly in fishes (Siltzer, 1943). The tissues involved may be sensitive to the hormone until they are fully differentiated, at which time they might lose this sensitivity. Gormann and Born (1908) have attributed a maturational effect to thyroxine, including effects on the integument, skeleton, and gonads. It is tempting to speculate that thyroxine may affect the growth and differentiation of the bones involved in the migration of the mouth of the sucker as well as in the metamorphic changes in
other tissues, such as the gut. Certainly, this explanation is highly speculative, and raises questions which are unanswerable at present.

Environmental effects may also be important in the metamorphosis of catostomids. Variations in temperature, day length, and iodine concentration are discussed in the appendix. At present, insufficient data are available on these factors to offer any real suggestion for their effect on the role of the thyroid gland in larval development of such fish as catostomids.
Figure 24. Diagram of anterior and posterior limits of thyroid gland, illustrating extension of the gland during larval period. (c = cartilage).
TABLE 3

COMPARISON OF THYROID GLANDS IN METAMORPHOSING AMPHIBIANS AND THE SUCKER

A. Relation between height of epithelial cells and extent of colloid.

<table>
<thead>
<tr>
<th>Stage of Metamorphosis</th>
<th>Xenopus laevis (Saxen, et al, 1957)</th>
<th>Sucker</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Epith.</td>
<td>% Colloid*</td>
</tr>
<tr>
<td>Premetamorphosis</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Prometamorphosis</td>
<td>57.8</td>
<td>27.7</td>
</tr>
<tr>
<td>Metamorphic</td>
<td>67.5</td>
<td>24.3</td>
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<tr>
<td>Climax</td>
<td>90.8</td>
<td>5.7</td>
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<tr>
<td></td>
<td>67.7</td>
<td>26.0</td>
</tr>
<tr>
<td>Postmetamorphosis</td>
<td>50.0</td>
<td>42.9</td>
</tr>
<tr>
<td></td>
<td>45.2</td>
<td>45.9</td>
</tr>
</tbody>
</table>

*Other tissues between the follicles make up the remaining percentage.

B. Change in epithelial height and colloid level produced by anterior lobe pituitary extract in Triturus torosus (after Uhlenhuth, 1945).

<table>
<thead>
<tr>
<th>Dose of ALPE in mg</th>
<th>Colloid Level Difference Between Exp. &amp; Control</th>
<th>Epithelial Height in microns</th>
</tr>
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<tbody>
<tr>
<td>100 daily</td>
<td>76%</td>
<td>7.1</td>
</tr>
<tr>
<td>30 every two days</td>
<td>37%</td>
<td>4.5</td>
</tr>
<tr>
<td>15 &quot;</td>
<td>11%</td>
<td>2.6</td>
</tr>
</tbody>
</table>

(Continued)
TABLE 3  
(continued)

C. Relation of epithelium.

<table>
<thead>
<tr>
<th>Stage of Metamorphosis</th>
<th>Pseudacris triseriata</th>
<th>Rana catesbiana</th>
<th>Rana palustris</th>
<th>Sucker</th>
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<tr>
<td></td>
<td>(after Etkin, 1936)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Premetamorphosis</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>**</td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prometamorphosis</td>
<td>**</td>
<td></td>
<td>**</td>
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</tr>
<tr>
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<td>***</td>
<td>**</td>
</tr>
<tr>
<td>Prometamorphosis</td>
<td>***</td>
<td>**</td>
<td>***</td>
<td>**</td>
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<td>Metamorphic Climax</td>
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</tr>
<tr>
<td>Postmetamorphosis</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

* Squamous  
** Low cuboidal  
*** Cuboidal  
**** High cuboidal  
***** High cuboidal to columnar

Note: Etkin included other criteria for these ratings, but only the epithelial shape allows comparison.
TABLE 4

CHANGES IN HISTOLOGY OF THE THYROID GLAND OF TWO SPECIES OF FISH

A. Change in the ratio of epith. vol./ follicle vol. after treatment with thiourea at 20°C in Phoxinus laevis (after Fortune, 1955).

<table>
<thead>
<tr>
<th>No. of days of treatment</th>
<th>Thiourea treated</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>.37</td>
<td>.50</td>
</tr>
<tr>
<td>10</td>
<td>.69</td>
<td>.24</td>
</tr>
<tr>
<td>24</td>
<td>1.00</td>
<td>.50</td>
</tr>
</tbody>
</table>

B. Actual epithelial cell height in Salmo gairdneri during the parr-smelt transformation. (After Robertson, 1948).

<table>
<thead>
<tr>
<th>No. of fish</th>
<th>Parr</th>
<th>Transforming Smolts</th>
<th>Smolts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epith. Ht. Ave. in microns</td>
<td>5.45</td>
<td>8.19</td>
<td>8.33</td>
</tr>
</tbody>
</table>
### TABLE 5
COMPARISON OF EH/FD RATIO IN ROUND AND OBLONG FOLLICLES

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ROUND:</td>
<td>34</td>
<td>37.0</td>
<td>2.8</td>
<td>0.16</td>
<td>1.12</td>
</tr>
<tr>
<td>OBLONG:</td>
<td>34</td>
<td>34.1</td>
<td>2.6</td>
<td>0.16</td>
<td>2.00</td>
</tr>
</tbody>
</table>

### TABLE 6
COMPARISON OF EH/FD RATIO IN ANTERIOR AND POSTERIOR FOLLICLES

<table>
<thead>
<tr>
<th></th>
<th>Number Measured</th>
<th>Average Diameter (in microns)</th>
<th>Average Epith. Ht.</th>
<th>EH/FD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANTERIOR:</td>
<td>30</td>
<td>34.5</td>
<td>2.7</td>
<td>0.17</td>
</tr>
<tr>
<td>POSTERIOR:</td>
<td>30</td>
<td>34.3</td>
<td>2.7</td>
<td>0.17</td>
</tr>
</tbody>
</table>
### Table 7
**Comparison of EH/FD Ratio in Large and Small Follicles**

<table>
<thead>
<tr>
<th>Number Measured</th>
<th>Average Diameter (in microns)</th>
<th>Average Epith. Ht.</th>
<th>EH/FD</th>
</tr>
</thead>
<tbody>
<tr>
<td>LARGE: 30</td>
<td>52.0</td>
<td>3.4</td>
<td>0.13</td>
</tr>
<tr>
<td>SMALL: 30</td>
<td>13.7</td>
<td>2.0</td>
<td>0.30</td>
</tr>
</tbody>
</table>

### Table 8
**Comparison of EH/FD Ratio by Stage and by Follicular Size Group**

<table>
<thead>
<tr>
<th>Average Diameter of follicles (in microns)</th>
<th>Number Measured</th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
<th>Stage 4</th>
<th>Stage 5</th>
<th>Stage 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-20</td>
<td>94</td>
<td>0.36</td>
<td>0.30</td>
<td>0.24</td>
<td>0.29</td>
<td>0.28</td>
<td>0.26</td>
</tr>
<tr>
<td>20-34</td>
<td>213</td>
<td>0.29</td>
<td>0.25</td>
<td>0.19</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>34-50</td>
<td>56</td>
<td>----</td>
<td>----</td>
<td>0.18</td>
<td>0.13</td>
<td>0.14</td>
<td>0.16</td>
</tr>
<tr>
<td>50-80</td>
<td>18</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td></td>
<td>0.12</td>
</tr>
</tbody>
</table>
Figure 25. Relationship of EH/FD ratio to size of follicle.

![Graph showing relationship between EH/FD ratio and size of follicle.]

Figure 26. Relationship of EH/FD ratio to stage of fish in two size groups of follicles.

![Graph showing relationship between EH/FD ratio and stage of fish for two size groups of follicles.]

Size group of follicles (in microns) in stage 6
1. Six arbitrary stages encompassing the metamorphic period of larval suckers are described. These are based on degree of fin development, nostril division, coiling and elongation of the gut, and migration of the mouth from a terminal to a ventral position.

2. The thyroid gland of each of the six stages is also described.

3. In all stages it consists of unencapsulated follicles scattered in mesenchymatous connective tissue along the ventral aorta near the bases of the first and second aortic arches. The arteries are arranged in a typically teleostean fashion. A thyroid artery supplies the posterior region of the gland while branches from the thyro-spiracular artery supply the anterior region. An extensive lymph and venous plexus is located in the anterior thyroid region. The inferior jugular vein is most prominent in the posterior region which has practically no lymph spaces. The thyroid follicles are intimately associated with the veins, lymph spaces, and capillaries.

4. The number of follicles increases greatly during metamorphosis, as does the size and extent of the gland.

5. Activity of the gland is assessed by the ratio of epithelial height to follicular diameter, as well as by other histological criteria.

6. The premetamorphic thyroid follicles are small, and possess primitive epithelial cells with relatively little cytoplasm. In succeeding stages, the epithelial height increases slightly, in keeping
with an increase in the relative amount of cytoplasm, average nuclear length, and granularity and vacuolization of the colloid. Colloid content also increases steadily, but to a greater degree. Epithelial cells are variable in height, but typically low cuboidal to cuboidal throughout.

7. A gradual decrease in the EH/FD ratio is interpreted as reflecting a period of colloid storage. Other factors suggest a gradual, slight increase in activity throughout metamorphosis. This increase is never great enough to produce a net loss of colloid, nor does there appear to be a histological crisis at metamorphic climax.
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APPENDIX

REPRODUCTION AND HATCHING

Hatching times are important to note because thyroid activity seems to be linked with temperature and photoperiod (Barrington, 1963). In western Montana, *Catastomus catastomus* and *C. macrocheilus* spawn in the spring. A comparison of present collection data with Stewart's (1929) timetable for early development of *Catastomus cormorsoni* suggests that peak spawning occurs in late May and early June. Stewart states that 15-20 days was a typical hatching time, and found yolk absorption complete in 6-8 days after hatching. Stage one of this study contains the last bit of yolk. Because this stage appeared in greatest abundance in collections in late June and early July, the total of 24-28 days required for *C. cormorsoni* to absorb the yolk would place peak spawning of the western Montana suckers at about the first week of June. This, of course, assumes similar developmental times for the three closely related species. Also, it assumes that the collections were reasonably qualitative. April and May had previously been suggested as probable spawning times for these fish (Weisel, 1957). There is undoubtedly a variability of spawning times from year to year, however. High water levels from snow runoff are necessary to back up the water into the sloughs used for spawning by the suckers. The time of this runoff varies considerably.
ENVIRONMENT AND BEHAVIOR

Because iodine content, water temperature, and photoperiod have been shown to affect the appearance and function of the thyroid gland in teleosts (Natty, 1960; Swift, 1960), a description of the changes in the slough is given here. Changes in behavior of the larval fish are described simultaneously.

The slough was connected at both ends with the main river until late July. As the water receded these connections were disrupted. Although there was no surface connection after this, seepage did occur in the upper end, as evidenced by continuing low water temperatures and several patches of white sand on a bottom that was otherwise thoroughly silted. Further drop in water level divided the slough into three sections.

The fish were first located in the lower section, which was separated from the middle section by an old beaver dam. Here, the water was the most shallow, being about three feet in depth where the fish were found. The fish were grouped into large, irregular schools which swam just beneath the surface. When frightened, they arrayed themselves in ranks and raced away from the source of disturbance, but made little attempt to hide among the ample aquatic vegetation. This behavior was quite different from fish collected in later post-metamorphic stages. Older larvae were more individualistic, swam further beneath the surface and among the vegetation, and took cover when frightened by hiding in the depths near the bottom. Later collections of juvenile fish were more difficult to obtain because
the fish often lurked near the bottom.

As the water receded and separated the lower from the middle section, numerous fish were trapped in the lower section which eventually dried up completely. Others traversed the narrow connection into the middle section, where no prolarvae had previously been located. For a short period before the lower section dried up, fish were located in both of these sections. At this time, the upper section was still connected to the middle section, but the fish remained in the latter where the temperature was warmer. Water to the depth of three feet was present in the middle section at this time, but the fish selected the sun-warmed shallows at the lower end and around the edge. Numbers of fish crossed the shallows between the middle and upper sections before the water had dropped enough to separate them. As was the case in the lower section, the middle section was isolated, and numerous fish were trapped and presumably killed as this section dried up completely.

Parts of the upper section were still about three feet deep at this time in the upper end where seepage was taking place. At no time did the upper section dry up completely, although the receding water reduced its area considerably. Fish preferred the warmer areas away from the seepage points until such time as the temperature of this upper end of the upper section warmed by virtue of the drop in water level. Behavior of late stages taken from this section was varied, with fish seeming to seek sunlit areas at times, and at other times hiding among aquatic plants and sunken trees. The water temperatures of the three sections during the collections are graphed
(Fig. 27). It may be that diminishing water levels in a situation like this result in an increase in relative natural iodine concentration. No data were taken on this.
Figure 2. Water temperature of the three sections of the slough during collections.