

STUDIES IN THE CONTROL OF  
PIGMENT CELLS AND LIGHT  
REACTIONS IN RECENT  
TELEOST FISHES

PART 1. MORPHOLOGY OF THE  
PINEAL REGION

PART 2. REACTIONS OF THE PIGMENTARY  
SYSTEM TO HORMONAL STIMULATION

PRISCILLA RASQUIN

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## INTRODUCTION

AMONG THE VERTEBRATES, the fishes are conspicuously affected in both their behavioral and physiological reactions by the amount and duration of exposure to light. The present two studies are concerned with the morphological structures other than the eyes, which exist in teleosts, that appear to be directly concerned with their reactions to light, namely, the pineal complex and the pigment cells. Breder and Rasquin (1947, 1950) have already indicated that the degree of pigmentation over the pineal area has an effect on the behavior of fishes, those with the most exposed regions being the most positively phototropic, and vice versa, although the tropisms are greatly modified by vision.

Coloration of teleosts in relation to background matching has been explored by numerous investigators. Nevertheless, the work on the control of changing pigmentation by the nervous and hormonal systems has been confined to a relatively few species, and these are mainly fresh-water or euryhaline types that are more or less easily maintained under laboratory conditions. The present report describes the pigmentary reactions to administered hormones by various species, including both fresh-water and marine forms, and also describes the morphology of the pineal re-

gions in relation to pigmentation and other light-obstructing features.

The observations described herein were accumulated over a period of several years, and acknowledgment of help is due many people for aid in the compilation of behavioral data and statistics, histological techniques, photography, and the collecting and identifying of specimens. Dr. C. M. Breder, Jr., was closely concerned throughout the course of the work, giving advice and suggestions. He helped with the experiment on effects of light and darkness on glycogen content of the pineal organ and was also responsible for the identification of all the species involved in the report. Dr. Phyllis H. Cahn assisted in many ways. Mr. Christopher W. Coates, of the New York Zoological Society, kindly made arrangements for the obtaining of *Phoxinus*. Many of the people who served as technicians are now scattered in various academic institutions. They include Dr. Libby R. Friedman, Miss Frances Radish, Miss Louise Stoll, Mr. Leonard Grosso, and Mr. Donn Rosen.

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## PART 1. MORPHOLOGY OF THE PINEAL REGION

### REVIEW OF THE LITERATURE

MUCH CONFUSION about the pineal organ that exists in the pertinent literature has its origin in a number of things, among which are ignorance of the embryological development of the organ, inconsistency in nomenclature, and conflicting ideas of function. Embryologically, in vertebrates, two evaginations are formed from the roof of the diencephalon, approximately in the midline. The more anterior of these is the paraphysis and the more posterior the epiphysis. In adult vertebrates, the paraphysis becomes the parietal organ and the epiphysis becomes the pineal organ; the two structures are not homologous (Tilney and Warren, 1919).

The anlage of the parietal organ appears to be absent in elasmobranchs, and there is some uncertainty as to whether or not it makes a transitory embryological appearance in amphibians, birds, and mammals. However, Kappers (1957) has recently described in *Scylliorhinus caniculus* an inconstant, variably shaped, rudimentary anlage of a true paraphysis found during embryonic stages but disappearing in the adult. In the cyclostomes, both the pineal and parietal organs become differentiated into eye-like structures situated close together in the median line beneath the skull on the dorsal aspect of the head. The cyclostomes represent the only class in which the pineal organ develops into a structure resembling an eye. In the teleosts, the development of the paraphysis is suppressed, and only the pineal organ becomes differentiated. In the reptiles, the opposite condition obtains, that is, the pineal is suppressed, and the paraphysis is differentiated into a parietal organ, often with retina and lens-like structures. It is not uncommon, however, to hear the words used synonymously, to read of the parietal organ of teleosts. The structure of the two organs is very different, the pineal of teleosts having none of the characteristics that make the parietal organ so like an eye.

A description of the morphology and histology of the teleost pineal organ and its asso-

ciated brain structures, as well as a history of the early literature on the subject, can be found in Studnicka (1905) and in Tilney and Warren (1919). In general the pineal organ consists of three parts: an end vesicle, a stalk, and an indistinct proximal portion or pedicle. Variations in these three parts ultimately make for vast differences in the structure in individual species. The proximal portion is closely associated with the brain and is usually not well defined. The stalk may be short or long and usually contains a lumen, although a solid stalk has been described for some species. The lumen of the stalk is continuous with the lumen of the end-vesicle. Nerve fibers have been described as traversing the length of the stalk and pedicle, connecting the end-vesicle with the habenular commissure, and fibers of this "pineal nerve" have been identified in the posterior commissure. The lumen of the stalk has also been described as being continuous with the third ventricle, but, according to other recorded observations, neither of these particulars holds for all teleosts. No nerve tract was discovered in *Argyropelecus hemigymnus* by Handrick (1901), and Terry (1910) could find no connection with the third ventricle in *Opsanus*, although a lumen was present in both stalk and end-vesicle. Rudimentary organs were described for *Syngnathus acus* and *Hippocampus spinosus* by Studnicka (1896).

The end-vesicle may be so small as to resemble hardly more than a widened blind-ending of the stalk, or it may be so specialized as to form a widespreading, umbrella-like structure over the top of the brain, extending from midway of the frontal lobes posteriorly to midway of the optic lobes. In the case of these large pineals, the walls of the end-vesicle are usually invaginated and convoluted, so that in sections many cords of cells are seen apparently surrounding numerous lacunae which are, in fact, all parts of the same lumen. This highly specialized pineal organ is common among surface-dwelling



fishes and is well represented in the families that make up the Clupeoidei and the Synentognathi.

In some species of teleosts, a foramen is present in the roof of the skull over the pineal area. The organ itself is found in the space beneath the connective tissue that connects the walls of the foramen. This structural complex is found in *Callichthys asper*, *Callichthys littoralis*, *Doras*, *Clarias*, *Loricaria* (Dean, 1891), *Argyropelecus hemigymnus* (Handrick, 1901), and *Astyanax mexicanus* and its blind cave derivatives (Breder and Rasquin, 1947). Sagemehl (1885), Gregory (1933), and Gregory and Conrad (1938) describe an open space in the skulls of several species of the Ostariophysi. There is also evidence that it is present in some larval forms, although it is not shown by the adult fish (Hill, 1894).

Von Frisch (1911a, 1911b), Scharrer (1928), and Breder and Rasquin (1947) have shown that the pineal organ of fishes is associated with pigmentation changes and with the responses of the animals to light stimulation. Although the organ appears glandular in part, sensory cells have been described in it, together with nerve fibers connecting the organ with other parts of the brain (Studnicka, 1905; Hill, 1894). It was therefore considered important to study the structure of the tissues overlying the pineal body in various species—those tissues that lie between the receptive organ and the stimulus of incident light. These tissues are, proceeding in order from the pineal distally, the meninges, part of the cartilaginous skull or chondrocranium, the bony skull, the dermis, and the epidermis. In some species a layer of fat is found between the meninges and the chondrocranium, and in others a heavy layer of muscle lies between the bony skull and the dermis. The massing of a considerable depth of tissue over the pineal does not necessarily indicate that the area cannot be stimulated by incident light, although it certainly diminishes the intensity of the stimulus.

Although Sterzi (1902) has asserted that fishes are provided with only one connective-tissue membrane covering the brain, in all the fishes observed at least two were encountered. Studnicka (1905) and Tilney and Warren (1919) also mention the existence of two membranes. The inner membrane cor-

responds to the pia mater of higher vertebrates and is closely applied to the surface of the brain. The outer membrane, corresponding to the dura, is thicker in construction and is often heavily invested with chromatophores. All forms of pigment cells are found in this outer meninx in various species of fishes, although all types are not present in every species. No fish of any species was found to have the meninges completely devoid of chromatophores. In some cases melanophores alone are present, but this condition appears to be confined to those fishes in which the brain is buried deep within the head, covered with heavy musculature. In fishes in which erythrophores are present in the dermis, erythrophores are also found in the dura, although they are not numerous. Usually xanthophores are abundant, and iridophores are also numerous, occurring both singly and in chromatosomes with melanophores and xanthophores. Leucophores are found in the dura of those fishes in which they are also found in the dermis. This covering of the brain with pigment cells has the result of protecting it from light, and it is significant that the area above the pineal region is usually deficient in chromatophores. In some of the exceedingly translucent fishes, as in *Carapus*, the brain and part of the spinal cord, invested with melaniridosomes, form a black line on the dorsal aspect of the head, visible to the naked eye. Figure 1 of plate 1 shows the brain and nerve cord of *Carapus bermundensis* covered with melanin and guanin. A small window, devoid of chromatophores is seen over the pineal region.

Plate 1, figure 2, is a photomicrograph of a section through the skin of the head of *Tylosurus raphidoma*, showing a chromatosome composed of one melanophore, the dendritic processes of which surround several iridophores. It can readily be seen how dispersion of granules in the cell processes of the melanophore can conceal the brilliance of the guanin in the cuboidal iridophores.

Early in the course of this work, it was noted that fishes could be divided conveniently into three broad groups in regard to the structures that cover the pineal area. The fishes of the first group possess a permanently translucent or semitransparent covering over the pineal area which permits the almost

unobstructed entry of light. The second group comprises fishes in which the pigment cells of the dermis and epidermis are so distributed that, by dispersion or concentration of the pigment granules, the pineal area can be either exposed to or protected from light. The third group comprises fishes in which the

pineal area is permanently covered with more nearly opaque structures of muscle, skull, and dense pigmentation (Breder and Rasquin, 1947). In the following descriptions, the various fishes that were examined in detail are grouped for convenience under these three headings.

## MATERIALS AND METHODS

In the present Part 1, which concerns the pineal anatomy, the fishes are arranged in three groups as above described. Within each group, the fishes have been arranged in order from those with the most specialized pineal to those with the least specialized organ. This grouping according to morphological characteristics of the pineal region causes the list of fishes to be disarranged as regards taxonomic order. In Part 2, concerned with the reactions of the pigmentary system to injected hormones of either mammalian or synthetic origin, many of the same species are referred to again, in the customary taxonomic order.

The fresh-water fishes used were made available in the laboratories of the Department of Fishes and Aquatic Biology at the American Museum of Natural History in New York. The marine fishes were collected and studied at the Lerner Marine Laboratory at Bimini, Bahamas, British West Indies. All dissections were made on fresh material, because the loss of color in formalin-fixed specimens made positive identification of various tissues too uncertain. The use of 1 per cent urethane for anesthesia served to bring about maximum dispersion of the pigment granules in the chromatophores.

For gross histological study, the heads of the fishes were fixed whenever possible in Bouin's picro-aceto-formol, decalcified in the phloroglucin-nitric acid solution, embedded in paraffin, sectioned at 5 to 9 $\mu$  depending on the toughness of the tissue, and stained by means of Harris' hematoxylin and eosin and Masson's trichrome methods. Some of the large heads, such as those of the tuna and the bonito, were fixed in 10 per cent formalin, changed several times, and decalcified in the electric decalcifier. These heads were em-

bedded in both parlodion and paraffin before being sectioned.

Cytological and histochemical techniques were carried out on the pineals of several different species, but mainly on the fresh-water *Astyanax* and the marine *Atherina*, partly because of their availability in large numbers and, in the case of *Atherina*, because of the large size of the organ. Most of the histochemical tests were made on the pineal of *Atherina stipes* in which the organ is sufficiently visible under the dissecting microscope to be dissected away from its surroundings. It was possible to fix and section the gland of this species without having to resort to decalcification, which destroys the usefulness of the tissue for many histochemical methods. A special feature that contributes to the visibility of the *Atherina* pineal *in situ* is the presence of xanthophores in the vesicle which gives a yellow color to the body of the gland. The exact position of these cells is not known, their outlines are invisible in the fresh state, and the pigment is dissolved in the course of the histological techniques so that the cells cannot be identified in section.

The following cytological and histochemical techniques were employed on the pineal organs of *Atherina stipes*: Mayer's mucicarmine and dilute toluidine blue stains for mucin; gallocyanin after formalin fixation for Nissl substance; Sudan Black B and Baker's acid hematein for lipids; Bauer-Feulgen and Best's carmine for glycogen; PAS and paraldehyde-fuchsin stains for glycoprotein; and Bodian's silver protargol for nerves and nerve endings.

The following techniques were employed on the pineal organs of *Astyanax mexicanus*: Heidenhain's azocarmine after fixation with Maximow's fluid for purposes of comparison



with the cytological descriptions of Friedrich-Freksa (1932); Mayer's mucicarmine and toluidine blue for mucin; PAS, Gomori's aldehyde-fuchsin without previous oxidation, and Sokol's modification of Halmi's stain for glycoproteins; Bodian's silver protargol for nerves; and Baker's acid hematein for phospholipids.

In addition, some of the same techniques were tried on pineals of other species, and other techniques were also employed. Thus Bielschowsky's technique for nerve endings was used on the pineal organs of *Atherina*, *Strongylura*, and *Tylosurus*; PAS was used on immature *Cypselurus*; Gomori's chrome alum hematoxylin-phloxin, and gallocyannin were used on *Jenkinsia*. The results of the various technical procedures are discussed in the section on cytological details and can be found also under the headings of the various species.

Some experimental work was conducted, and the description of the materials and methods used in each case is found in the sections that describe the experiments. An attempt was made to discover the results of epiphysectomy on the light responses of

*Astyanax*, an experiment was conducted to determine the effects of sojourns in light and darkness on the glycogen content of the pineals of *Cypselurus* juveniles and *Atherina* adults, and pineal injections were made in *Cyprinodon* to discover what the result might be on the pigmentation. These experiments are recorded under their proper headings.

Sokol's modification of Halmi's paraldehyde-fuchsin stain (Sokol, 1953) proved very useful in the tracing of the pineal stalk to its communication with the third ventricle. Distortions that result from the histological techniques occasionally crowd the parts of the brain together, so that it is impossible to discriminate between the pineal stalk tissue and its surroundings. The cell processes in the lumen of the stalk are easily recognized in sections stained in this way because of their reaction to the paraldehyde-fuchsin which is in strong contrast to surrounding tissues. Unfortunately many of the pineals used in this report were sectioned before the usefulness of the stain was discovered, and the technique is not successfully applied to material that has already been stained.

## MORPHOLOGY OF THE PINEAL REGION IN VARIOUS SPECIES

### FISHES WITH EXPOSED PINEAL REGION

#### *Jenkinsia lamprotaenia* (Gosse)

A transparent, window-like area is present in this species over the pineal area, larger in proportion to the size of the brain than in *Atherina*. (See pl. 1, fig. 3.) This club-shaped area can be seen with the naked eye in the living fish. The clear area reveals the posterior half of the forebrain and part of the optic lobes which can be clearly seen through the top of the head under the dissecting microscope. Some specimens show a few chromatophores in the dermis over this area; others show none. The head of *Jenkinsia* is naked of scales, and the skin is closely applied to the bone of the completely transparent skull. It can be clearly seen that immediately beneath the skull lies the membranous covering of the brain that is everywhere heavily invested with iridosomes except in the area over the pineal region. The meninges are

devoid of chromatophores under the entire window-like area.

In sections stained with Masson's trichrome connective-tissue stain, the pineal is seen to be a large, flat organ extending anteriorly three-quarters of the way forward over the frontal lobes. It is extremely convoluted, and the lumen is consequently divided into many small, flat spaces. Two kinds of nuclei are obvious, one large and vesicular, one smaller and more highly chromatic. The cells are all small and more or less cuboidal. The stalk inclines anteriorly from the pedicle, so that all the end-vesicle is anterior to and thus not covered by the endosteum. The gland spreads over the frontal lobes but does not extend posteriorly over the optic lobes. The lumen of the stalk was visible in section for about half of its length, extending posteriorly from the vesicle. In Bouin-fixed sections stained with PAS, cell boundaries are indistinguishable. All the cells appear to have

tiny, pinpoint spots of positive reaction, which indicates the presence of glycogen. In occasional places the cell processes protruding into the lumen take a pale, diffuse pink. Undecalcified material, fixed in Helly's fluid and stained with Gomori's chrome alum-hematoxylin and phloxin, was completely negative for stained neurosecretory material. Formalin-fixed sections, stained with gallocyenin were negative for the presence of Nissl substance.

***Sardinella macrophthalmia* (Ranzani)**

No scales are present on the top of the head. A few melaniridosomes are found in the dermis together with xanthophores. The meninges are clearly seen from the top of the head without dissection. They are heavily invested with iridophores, xanthophores, and melanophores. A large, unpigmented area lies over the pineal region. This is T-shaped, the main stem of the T extending posteriorly nearly to the posterior border of the optic lobes. The cross bar of the T extends the full width of the brain. The pineal area in this species obviously cannot be covered by dispersion of dermal chromatophore granules. The skin is closely applied to the bone of the transparent skull. When the skull is removed, it splits in two down the midline, revealing the thin, cartilaginous endosteum which covers and is the same shape as the T area.

In section, the vesicle is seen to be large, with extremely convoluted walls. Most of the body of the vesicle lies anterior to the chondrocranium under the bony skull and over the frontal lobes of the brain. The same two kinds of cells are noted in the epithelial lining of the vesicle, and cell processes almost completely fill the lumen. The stalk is long and inclined anteriorly from the short pedicle. There is a wide lumen in the stalk near the vesicle which in the sections appears to be closed about halfway down the stalk. The lumen is lined with pineal cells. Numerous capillaries are found in the walls of both vesicle and stalk. The stalk is closely applied to the posterior wall of the dorsal sac.

***Atherina* (*Hepsetia*) *stipes*  
(Müller and Troschel)**

A transparent, window-like area is seen in the head over the region of the pineal. This

area is so transparent that, without any dissection, the blood vessels that supply the brain are readily visible, as are the exposed parts of the frontal and optic lobes. Only a few chromatophores are seen in the integument covering this transparent area, and these are melaniridosomes. A single scale covers the window-like area. When this is removed the dermis can be seen to be closely applied to the bone of the skull, which is nearly transparent. When it is removed, a part of the chondrocranium, the endosteum, is revealed, covering the T-shaped, window-like area. Removal of the skull leaves the optic lobes and forebrain uncovered except for a heavy opaque layer of chromatophores with which the meninges are invested. The opacity appears to be largely due to crystalline guanin contained in iridophores and melaniridosomes. The transparent, T-shaped area over the pineal region is sharply delimited by the arrangement of these chromatophores in the membrane that covers the rest of the brain. The chondrocranium branches laterally at the top of the T-shaped area and connects with cartilaginous elements immediately medial to the eyeballs. The meninges pass under the chondrocranium and in the transparent area contain no guanin but a few melanophores and fairly numerous xanthophores. The vesicle of the pineal also contains xanthophores. A layer of fat lies over the forebrain but is absent over the pineal region and optic lobes.

Plate 2, figure 1, is a photograph of a transverse section through the pineal area of *Atherina*. The lumina on each side of the stalk are parts of the single lumen of the dorsal sac, to the posterior wall of which the pineal stalk is closely applied. The stalk of the pineal is attached in the median line to the habenular commissure, immediately anterior to the large dorsal sac which is formed by a part of the roof of the third ventricle. No differentiation of a proximal portion or pedicle is seen. The stalk is lined with cuboidal epithelium, and, between the two layers of cells that form the walls of the stalk, fibers are visible running lengthwise of the stalk. These do not take the fast green stain when Masson's connective tissue stain is used and are therefore not collagenous. They have previously been identified as nerve fibers

but all do not stain strongly with Bodian's protargol technique. They are probably composed of both nerve and reticular fibers. The stalk is fairly short and widens out into the end-vesicle before reaching the level of the most dorsal aspect of the optic lobes (pl. 2, fig. 1).

In this species, the end-vesicle is a large organ spreading out anteriorly, posteriorly, and laterally from the stalk. The anterior border is found about midway of the frontal lobes, and the vesicle reaches posteriorly to midway of the optic lobes. It contains a lumen, but the walls are so infolded that the cells assume the structure of cords, with the lumen seeming to be like sinusoids between them. The entire vesicle is surrounded by a thin, collagenous, connective-tissue capsule. The organ has a connective-tissue stroma, and reticular fibers are prominent. When seen in transverse section, these resemble granules.

The cells have very little cytoplasm, and cell boundaries are difficult to determine. In Bouin-fixed, Masson-stained sections, the cytoplasm takes a grayish stain in contrast with the red nuclei, and seems to be homogeneous. Three kinds of cells are distinguished: one with round nuclei, one with oval nuclei, and one with extremely large nuclei and heavily stained granules in the cytoplasm. This last cell is very rare. Mitotic figures are occasionally seen.

In Bouin-fixed sections stained with PAS, small positive droplets of varying sizes are seen in the cells. Rarely, a cell is seen to be crowded with these. In some cells a thread-like, short strand is positively stained; in other cells a positively stained network resembles a Golgi apparatus. In occasional areas the cell processes in the lumen are faintly positive but are not so darkly stained as the positive points in the cells. None of the droplets appear in the lumen. When the Bauer-Feulgen stain for glycogen is used, the positive reaction seems more definite. Positive patches of various shapes and sizes are mostly intracellular. Those outside the cells were not found in the lumen in these sections. The cells most laden with positive droplets are those in the most dorsal part of the vesicle, nearest the skull. In a second similarly stained pineal, the cells were seen to be

fairly full of positive material, some in dots, some in rods and long strings, but all intracellular.

In formalin-fixed pineals, stained with gallocyanin, two kinds of nuclear staining occur. In one, the large, round nuclei are light and vesicular; in the other, elongate or small round nuclei are much more chromatic. The latter are more numerous than the vesicular nuclei. Nothing has the slightest resemblance to Nissl substance in any of the cells. Formalin is a poor fixative for the pineal, as it shrinks the cells towards the capsule wall and makes the lumen seem much larger than it actually is.

In pineals stained with Bodian's silver protargol, fibers are not particularly noticeable in the stalk or in the commissure. Some heavily blackened fibers can be seen in the base of the stalk but cannot be followed for any appreciable distance. Fibers are numerous in the vesicle running laterally, or anteroposteriorly. Some large cell bodies stain deep gray. Plate 7, figure 5, is a photomicrograph of an *Atherina* pineal stained with Bodian's technique, and plate 7, figure 6, is a higher magnification of the same section. Small, free, nerve endings can be seen, in many cases close to the nuclei or the cells of the parenchyma.

Plate 7, figure 3, is a photomicrograph of an *Atherina* pineal stained with Bodian's technique, showing ganglion cells and their connections in the ventral aspect of the vesicle where it narrows down to become the stalk. The black blotch on the left is a melanophore in the concentrated condition lying in the meninx under the vesicle. This was the only part of the pineal organ in which these ramifications of ganglion cells were seen, but the silver impregnation techniques are so capricious that it is possible the ganglion cells exist in other parts of the vesicle, remaining unstained by the silver protargol.

In order to find out whether lipids were present in the pineal cells, three *Atherina* pineals were fixed in cobalt-formalin. They were mordanted in 3 per cent potassium dichromate and embedded in paraffin. The sections were stained with Sudan Black B and mounted in glycerin. Bluish black granules were seen in the cytoplasm of the pineal cells in all three organs. The granules were

not numerous and probably were mitochondria. This probability is confirmed by pineals stained with Baker's acid hematein for phospholipid. After this method, most of the cells contained some positive material. The positive particles were round rather than rod-shaped, and mostly were collected in small groups.

***Carapus bermudensis* (Jones)**

The skin of the entire fish is devoid of scales, and most of the animal is exceedingly translucent. There are no chromatophores in the skin over the top of the head, and the skin is closely applied to the skull. The meninges covering the brain are heavily invested with melanophores except for a small area over the pineal region which is free of pigment. The body musculature is inserted posterior to the medulla, and the brain with its dark covering can be seen in its entirety from the top of the head without dissection (pl. 1, fig. 1). Five specimens were studied; all were adults. All had the clear space over the pineal area of the brain. The melanophores are very large and when in the dispersed condition are capable of covering a large area. Two melanophores are seen in the dermis on each side of the anterior part of the head, which appear to cover the olfactory lobes. Smaller cells are seen more abundantly over the chin and extreme fore part of the head.

Compared with other species, *Carapus* has relatively few chromatophores. The reddish color of the fish is due to the color of the blood that shows through the transparent musculature, and the color is reinforced in places by erythrophores. Scattered melanophores and erythrophores are found along the midline of the dorsal surface, but these number only in the dozens. A dark horizontal line is formed by a few melanophores over the insertion of the body musculature. A few melanophores are seen on the ventral surface also. The caudal fin contains erythrophores. A lateral stripe is made by patches of guanin that is in the form of large crystals, rather than in the more usual iridophore or melaniridosome. Guanin is also found in the iris and the covering of the eyeball. The darkest areas of the fish are caused by the melanophores in the meninges and the melanophores and iridophores present in the peritoneum.

Melanophores are also seen along the nerve cord and dorsal aorta. All these details are seen without dissection, through the transparent musculature.

In section, the epithelium covering the head is found to be extremely glandular in character. About half of the glands are obviously mucoid, filled with homogeneous material that stains with the fast green of Masson's trichrome stain. The remaining glands are filled with a granular substance deeply stained by acid fuchsin. The epithelial layer is closely applied to the skull; a very small amount of connective tissue lies between it and the bone. A large space filled with loose, granular, acellular material lies in the cranial cavity between the skull and the meninges. An occasional blood vessel traverses this material, and the pineal is found embedded in it. The meninges are heavily invested with melanophores with the exception of a small area over the pineal vesicle where they are absent. The pineal in this species is very small and compact. The walls of the vesicle are convoluted so that the lumen is difficult to distinguish, but the vesicle itself is a small one and lies directly above the insertion of the stalk between the forebrain and optic lobes without any anterior or lateral expansion. There are very few capillaries in the connective-tissue capsule. The stalk is long and extremely thin and fragile, consisting mainly of nerve fibers, with very few pineal cells present. The pedicle is short and broad.

**FISHES WITH CONTROLLABLE PINEAL COVERING**

***Strongylura notata* (Poey)**

The following description of gross anatomy was made from the dissection of a rather small, immature specimen which measured about 100 mm. in standard length. However, four others were examined later, and all showed the same characteristics except that the bone of the skull was much heavier in the older individuals. The standard lengths of these four fish ranged from 226 mm. to 384 mm.

The top of the head is naked of scales. The skin is quite thin and delicate and is closely applied to the skull. The dermis is generously supplied with melaniridosomes. When the skin is scraped away, the skull bone is seen



to be perfectly transparent. The brain beneath is covered with a dura that carries a dense concentration of chromatophores which are formed of combinations of melanophores and iridophores, and combinations of melanophores, iridophores, and xanthophores. A long, transparent space in the dura is free of pigmentation over the pineal area. This space is in the form of an isosceles triangle of which the base lies about midway of the forebrain and the apex is about midway of the optic lobes. By concentration of the dermal chromatophore granules of the top of the head, this area could be exposed for the admission of light. Under the high magnification of the dissecting microscope, the seemingly transparent dura is seen to contain a few scattered xanthophores.

Plate 2, figure 3, is a photograph of the dorsal view of the head of *Strongylura notata* after administration of adrenalin. Under the influence of this hormone, the melanophore granules become concentrated. This simple experiment demonstrates how the area over the pineal region can be cleared of any pigment obstructing the admission of light. In its light phase, the fish normally takes on this condition of pigmentation although perhaps in not so pronounced a manner.

In Bouin-fixed, Masson-stained sections, two kinds of cells in the pineal are well differentiated. One cell type has smaller, more highly chromatic nuclei than the other, and the cytoplasm is darker. These are possibly the supporting cells described by Friedrich-Freksa (1932), in as much as the processes within the lumen seem not to come from these cells. The processes in this species appear less fibrous than in others and have a more hyalin appearance. The cell types are not differentiated by different staining reactions, for both stain with ponceau, but the darker cells seem to have a more grayish cytoplasm.

The sections show that the pineal organ extends anteriorly to midway of the frontal lobes; the stalk inclines anteriorly from its base, and only a small part of the vesicle is found posterior to the plane of insertion of the stalk. The stalk is broad and lined throughout with pineal cells. The stalk has a lumen throughout its length, and this communicates with the third ventricle. A longitudinal section through the base of the pineal organ in

this species is seen in plate 2, figure 2. The narrow communication with the third ventricle is clear, and the short pedicle with fibrous walls is seen between the end of the stalk and the ventricle. The band of tissue closely associated with the pineal stalk on the right is the posterior wall of the dorsal sac.

In sections stained by Bodian's silver-protargol method, nerve fibers can be readily identified in the stalk and pedicle but cannot with any certainty be traced to the habenular commissure. Nerve fibers are also found in the capsule of the vesicle, and short branches of these appear to end freely among the pineal cells.

#### **Brachydanio rerio** (Buchanan-Hamilton)

No scales are present on the top of the head. A crescent-shaped translucent area is seen over the posterior part of the forebrain, between the forebrain and the optic lobes. This area is broader in the center over the pineal region and narrower at each side where it reaches the full width of the brain. When the chromatophores are in the dispersed condition, as they are when the animal is under the influence of urethane, the area over the optic lobes is a dense black, as the melanophores are numerous both in the dermis and in the meninges in this region. Melaniridosomes in the dura are clearly seen over the frontal lobes through the undissected top of the head. The skin is closely applied to the skull, and the skull itself is completely transparent. The body musculature is inserted posterior to the cerebellum. A few scattered melaniridosomes are seen in the dura over the pineal area, but the dura covering the rest of the brain is heavily invested with melanophores, melaniridosomes, and rare xanthophores.

#### **Phoxinus phoxinus** (Linnaeus)

There are no scales on the head. In the skin of the living specimen, the melanophores can be seen to cover the pineal area completely when the cells are in a dispersed condition. The skin and skull are sufficiently transparent to allow vision of the chromatophores on the meninges covering the optic lobes through the surface of the top of the head. The skin is closely applied to the skull; the body musculature is inserted posterior to the

cerebellum. When the skin is removed, the skull is seen to be completely transparent, and the meninges are heavily pigmented with melanophores and iridophores except for a transparent window over the pineal area. By a concentration of the pigment granules in the melanophores of the skin, the fishes could expose the pineal region, but they are not forced to leave the pineal area unprotected as does *Atherina* or *Jenkinisa*.

**Gambusia** sp.

The species is the one that is found in the salt water at Bimini. A single scale is found on the top of the head, which is transparent, so that the brain may be seen without any dissection. Melanophores in the skin can cover the pineal area if they are in a dispersed condition. Bouin-fixed, Masson-stained sections show that the pineal organ is fairly large, extending approximately one-fifth of the distance forward over the frontal lobes. The posterior part of the vesicle lies under the chondrocranium, and most of the vesicle extends posteriorly over the optic lobes. The stalk inclines anteriorly from its point of origin in the pedicle and is long and fairly thick, lined with a single layer of cuboidal cells. These cells are of two types, one with large round vesicular nuclei and clear cytoplasm, the other with smaller, more highly chromatic nuclei and muddy cytoplasm. The lumen of the stalk is open all the way down to the pedicle, but the opening into the third ventricle was not identified.

The end-vesicle is large, with extremely convoluted walls. The same two varieties of cells are represented in the epithelium, although they are larger than those in the stalk. The lumen is narrow and tortuous and is occupied by cellular debris. Capillaries are numerous in the connective-tissue capsule wall, between the capsule and the epithelium. The capsule is formed by a very thin, collagenous, tissue membrane. Fibers can be seen in the stalk.

Transverse sections were studied after they had been stained with Gomori's chrome-hematoxylin and phloxin method, and no sign of neurosecretory material was seen in the neighborhood of the pineal. PAS-stained sections showed the cellular debris to be a faint pinkish color. Formalin-fixed sections,

stained with gallocyenin, showed a complete absence of Nissl substance in any cells in the pineal.

**Lebistes reticulatus** (Peters)

A single scale is found on the top of the head, which is transparent, so that the brain may be seen without any dissection. The body musculature is inserted posterior to the optic lobes, so it does not cover the pineal area. The meninges are heavily invested with melanophores and iridophores. A small, irregularly shaped, clear space is seen in the meninges over the pineal area. This area can be fairly well covered by dispersion of granules in the dermal melanophores, but it is still faintly visible under the conditions of expansion caused by urethane anesthesia. The skull is thin, and the pineal organ can be seen under the dissecting microscope through the top of the head.

The stalk inclines anteriorly from the pedicle so that the entire vesicle lies forward of the boundary of the chondrocranium directly under the bony skull. It extends from about midway of the frontal lobes to the posterior part of the same lobes. The vesicle is flat, and the lumen is filled with cellular debris which is faintly blue-gray when stained with Heidenhain's azan. The same two types of nuclei are seen, one smaller and more hyperchromatic than the other. Cell boundaries are indistinguishable. The vesicle is not very large but merges imperceptibly with the stalk which is wide and lined for almost its entire length with pineal cells. Most of the stalk lies under the chondrocranium as well as the bony skull. Meninges are heavily pigmented but not in the pineal area. Pigmentation is seen in the sheath under the pineal vesicle at the periphery of the organ.

**Mollinesia sphenops** (Cuvier and Valenciennes)

The top of the head is covered with scales. Melanophores can be seen lying ventral to the pineal vesicle in the meninges. A few scattered melanophores are present in the dermis over the pineal area, but are much more numerous lateral to this area. Epidermal melanophores are extremely rare. The pineal vesicle lies directly beneath the skull and is rather large for the size of the fish. It extends from about midway of the forebrain

to the posterior edge and extends laterally over half of each lobe of the forebrain. Its walls are greatly convoluted and richly vascularized. The lumen is filled with cell processes and cellular debris. The main part of the vesicle lies anterior to the chondrocranium. The vesicle narrows to a thick stalk which is lined with pineal cells for at least half of its length. The stalk is long and ends in a short pedicle.

***Mollinesia latipinna* (LeSueur)**

Melanophores are numerous in the dermis and epidermis, and the skull is thin and transparent. In spite of the thickness of the chromatophores in the skin, the outlines of the brain can be seen covered by the meninges. These membranes are heavily invested with melanophores, and the blackness is relieved by points of light which indicate the presence of iridophores. A triangular space over the pineal gland is free of chromatophores. The body musculature is inserted posterior to the optic lobes and does not cover the pineal area.

***Belonesox belizanus* (Kner)**

When in the light phase, the top of the brain of this species is easily seen through the overlying tissues, reflecting light from the iridosomes of the meninges. The spot over the pineal is visible only under magnification but is devoid of meningeal chromatophores. The clear area is a broad, triangular spot, the apex of the triangle pointing anteriorly. In the dark phase, which can be brought on by urethane anesthesia, dispersed dermal melanophores completely cover the pineal area. The scales and epidermis are thin and closely applied to the skull, and, when scraped off the skull, the pineal area is clear and perfectly visible through the transparent bone.

The pineal lies close to the surface of the head, covered only by the bony skull, which is extremely thin in this region, and a thin connective-tissue dermis and epithelium. The whole vesicle lies anterior to the chondrocranium over the middle region of the forebrain. The stalk is long and inclined anteriorly from the small pedicle. A lumen is present throughout the length of the stalk, lined with pineal cells. The lumen of the vesicle is nearly obliterated by the numerous infoldings and convolutions of the wall. When Halmi's

stain is used, the cell processes are deeply stained with paraldehyde fuchsin. The proximal portion of the stalk or pedicle consists mainly of fibers, and the communication of the lumen with the third ventricle was not seen in the sections.

***Sphyraena barracuda* (Walbaum)**

Under urethane anesthesia, this fish darkens to almost black except for the ventral surface. The epidermal melanophores are numerous and, when in a dispersed condition, completely cover the heavy concentration of iridophores and melanophores that lie in the dermal layer beneath. The top of the head is without scales, and no translucent area is seen. Plate 2, figure 4, shows the completely opaque head of a barracuda under urethane anesthesia when all the melanophores were in a dispersed condition. However, when the fish assumes a pale phase, a fairly large rectangular area which is lighter than the surrounding skin can be seen over the pineal region. This light, rectangular area is more marked in immature specimens but can be detected in the large, adult forms.

The skin is closely applied to the skull, and dissection shows that the body musculature is inserted posterior to the cerebellum. The skull is dense and spongy over the midline, but lateral to the midline, after the skin has been removed, it is transparent enough to permit vision of the forebrain and optic lobes covered with meninges containing melanophores, iridophores, and some xanthophores in dense concentration. A pinkish area devoid of chromatophores can be seen through the spongy bone overlying the pineal area. On further dissection, the pinkish area is seen to be the pineal region not covered by any melanophores. There is a shallow fossa in the skull over this pineal area.

In section, the gland is seen to be fairly large, extending anteriorly about one-quarter of the way over the frontal lobes and posteriorly one-sixth or one-eighth over the optic lobes. The lumen of the vesicle is wide and virtually empty; the walls are not particularly convoluted. Two types of cells are apparent here also. The stalk is intimately associated with the posterior wall of the dorsal sac. In the photograph (pl. 3, fig. 2), which represents a sagittal section through

the pineal region, pigment cells can be seen in the meninges passing ventral to the pineal.

***Apogonichthys stellatus* (Cope)**

The skin of this fish is thin and transparent; the scales are deep set and adherent. Epidermal melanophores are large, with long, delicate, dendritic processes. Dermal melanophores are extremely numerous and vary greatly in size. Some are small, and some are so large that individual cells can be seen with the naked eye, especially in the head region. The deeper layer of the dermis carries relatively few melanophores and many iridocytes with which the entire body is sheathed. The head is devoid of scales. The transparent skin is, however, so heavily invested with melanophores that there is no translucent area over any part of the brain. The skin is separated from the bone of the skull by a thin layer of musculature. The muscles are inserted between the eyes. The low occipital crest rises from the skull at the anterior level of the forebrain. All bones of the skull are transparent. When the melanophores of the dura are in the concentrated condition, a narrow area devoid of chromatosomes can be seen over the pineal area and the latter half of the forebrain. Under the dispersed condition of the melanophores this area is well covered with melanin. The pineal body, which lies directly beneath the occipital crest, can be seen through the skull when the crest is removed. It seems possible that if the melanophores were all in a concentrated condition, light would be able to reach the pineal region. It is noteworthy that the pigmentary behavior of this fish under normal conditions corresponds to that of the blinded *Phoxinus* of von Frisch (1911a, 1911b), in as much as the coloration of the animals becomes light in the dark and dark in the light.

**FISHES WITH PERMANENT PINEAL  
COVERING**

***Astyanax mexicanus* (Filippi)**

The top of the head is devoid of scales. Melanophores as well as iridophores are plentiful, and there is no area less pigmented than another. The open foramen in the midline of the skull is closed with collagenous connective tissue and the pineal lies beneath,

most of the vesicle lying anterior to the bony bar which crosses the foramen about midway of its anterior-posterior axis.

In Bouin-fixed tissue, stained with hematoxylin and eosin, the pineal appears as a fairly small organ, with a single lumen and a few convolutions in the wall. Three kinds of nuclei are seen: one is large, round, and vesicular; the second is more oval and sometimes nearly fusiform in shape; and the third type resembles a connective-tissue nucleus. These last are most numerous near the capsule of the gland. Blood vessels are seen in the capsule. Cell boundaries are not distinguished, and the nuclei are in the distal parts of the cells, next to the lumen. The lumen is traversed by an irregular network made by cellular debris. In PAS-stained pineals, there is a positive reaction in the walls of the capillaries and in the capsule as well as in small spots within the cells. There is a slight positive reaction in the tips of some of the cells and in the material in the lumen. Intracellular material is not stained after the sections have been digested with saliva, which indicates that the material is glycogen. With Gomori's aldehyde-fuchsin method, the material within the lumen is stained slightly.

In tissue fixed in Maximow's fluid and stained with Heidenhain's azocarmine, the better staining of the basement membrane shows the walls to be much more convoluted than was indicated by means of other stains. The cells of the vesicle are bluish gray in this stain, and the debris in the lumen takes the same color and appears fibrous. Among the fibers are small hyalin bodies which take a faint orange or yellow stain.

Other details of the cytology of the pineal organ of this species can be found under the appropriate section of this report. The species was also used by Grunewald-Lowenstein (1956) for studies on the glycogen content of the organ.

***Eucinostomus gula* (Cuvier and Valenciennes)**

No epidermal melanophores can be seen. The top of the head shows no translucent area and is covered with scales. The dermis is heavily invested with melaniridosomes. The skull is divided anteriorly, making room for the folding-away of the maxillary processes. The muscles of the head are inserted on

each side of this division, anterior to the eyes. The occipital crest supporting the heavy musculature of the head rises from the skull at the posterior edge of the skull division anterior to the forebrain. As a consequence the whole brain, with the single exception of the olfactory lobes, is covered by thick layers of muscle. When the musculature is dissected away, the skull bone is seen to be thin and transparent, and the brain is visible through it. The dura carries dense concentrations of iridophores, melanophores, and leucophores, but the area over the pineal region is devoid of pigmentation.

In Bouin-fixed, Masson-stained sections, the vesicle of the pineal is seen to be fairly large but simple in structure, with few convolutions of the wall. A few cells with highly chromatic nuclei were seen in the lumen. The cytoplasmic processes are large, almost as large as the cell nuclei. The cells are more cuboidal than in most other species. The stalk is long and has a lumen. Pineal cells do not extend down to the pedicle. From a considerable distance dorsal to the pedicle the cells appear to be simple epithelial lining cells. Fibers are conspicuous in this area of the stalk.

#### *Gerres cinereus* (Walbaum)

The head is covered with scales. There are no epidermal melanophores, and the scales, when pulled out, come away clean without pigment cells. Dermal melanophores are numerous, mostly combined with iridophores to make melaniridosomes. No translucent area appears on the top of the head. As is the case in *Eucinostomus*, the muscles of the head are inserted anterior to the eyes, on each side of the skull division that provides space for the accessory maxillary structures. The occipital crest, rising from the skull and supporting the heavy musculature, is attached at the anterior end of the skull, forward of the eyes, at the division of the skull. The larger blood vessels in this very heavy musculature are provided not only with surrounding melanophores but with iridophores also. The bone of the skull is heavy and somewhat spongy but sufficiently transparent in places to allow the chromatophores of the meninges to show through. A layer of fat underlies the bone over the top of the brain. There is no

pigmentation in the meninges over the pineal body.

In section, the pineal gland is seen to be buried beneath heavy layers of scales, musculature, bony skull, and chondrocranium. It has a large vesicle, however, reaching from the posterior end of the frontal lobes to the anterior border of the cerebellum. The stalk comes up nearly vertically from the pedicle and then inclines posteriorly so that most of the vesicle lies posterior to the insertion of the stalk. The walls of the vesicle are greatly convoluted; the lumen is divided and subdivided into many flat spaces and contains much cellular debris (pl. 3, fig. 2). The connective-tissue capsule contains fairly large blood vessels. The stalk has a lumen throughout most of its length, but this is often difficult to distinguish because of the disintegrating material within it. In the photograph the broad clear band is the cartilage of the chondrocranium with part of the skull above it. This gives some idea of the depth at which the pineal organ is found, for musculature and integument are found above the skull.

#### *Pseudupeneus maculatus* (Bloch)

Scales cover the top of the head, and no translucent area is seen. Chromatophores are numerous, forming a complicated system of melanophores, xanthophores, erythrophores, and iridophores. The integument is closely applied to the skull only anterior to and between the eyes. A thin layer of muscle is found inserted in the region of the eyes. The occipital crest supporting heavy musculature arises from the skull immediately posterior to the frontal lobes. The muscle layer is therefore rather thin over most of the brain, and heavy musculature is found posterior to the anterior edge of the cerebellum. The meninges over the cerebellum, which is so deeply buried, carry only scattered melanophores, but the forepart of the brain, including the optic lobes and forebrain, is covered by the same membranes which here contain scattered melanophores and a heavy investment of guanine in the form of iridophores. A small space devoid of chromatophores lies over the pineal area. The bones of the skull are thin and so transparent that the foregoing details could be seen without removal of the skull. Examination of an immature specimen



showed essentially the same conditions.

Sections show that the vesicle lies entirely under the cartilaginous chondrocranium. In the juvenile specimen the vesicle is a large body containing a lumen, the dorsal wall of which is smooth while the ventral wall is extremely convoluted. Short cellular processes project into the lumen which is otherwise clear. The stalk is wide near the vesicle but narrows rapidly and for most of its length is composed mainly of fibers. The meninges which pass under the vesicle are heavily invested with melanophores. The vesicle is short and rather broad, not at all elongated along the anteroposterior axis; the whole organ lies over the midpart of the brain. The stalk is very long and inclines anteriorly from the short pedicle, closely applied to the posterior wall of the dorsal sac.

#### ***Betta splendens* (Regan)**

The head is covered with scales, and there is no translucent area over the pineal. The musculature is inserted immediately posterior to the eyes, so that the pineal area is also covered with muscle. The skull is thin and transparent, and the meninges are invested with melanophores and iridophores. No clear space in the meninges over the pineal organ was noted in the single specimen examined.

#### ***Cyprinodon baconi* (Breder)**

Bouin-fixed, Masson-stained sections show the pineal gland to be small; the vesicle contains a lumen undivided by the few infoldings of the wall. The vesicle is situated anterior to the end of the chondrocranium and is covered by two layers of heavy bone and dermis which contains chromatophores. Two kinds of cells are present. One has a vesicular, oval nucleus with a single large nucleolus. The second type, with more hyperchromatic nuclei, is nearly fusiform in shape in this species. Near the anterior end of the vesicle all the cells are very long; the nuclei are near the distal end, and the proximal ends of the cells are greatly attenuated, reaching back to the membrane. Cell boundaries are indistinct. A faint greenish tinge is seen in the cytoplasm of some of the cells, and this is also seen in some of the cellular elements protruding into the lumen. All the cells are more

cuboidal at the posterior part of the vesicle as it nears the stalk. Mitotic figures are seen in the epithelium.

The stalk is very thick, lined by high columnar cells, and the lumen is in open communication with the third ventricle.

In sections stained with PAS, the capsule and capillary walls are positively stained. Many of the cells have small spots of positive reaction within them. These resemble dots, or small threads, and sometimes the nucleus is outlined by them. A rare cell has several droplets of varying size. The use of the chrome hematoxylin-phloxin method did not reveal any neurosecretory material.

Several adult *Cyprinodon* of both sexes were implanted peritoneally with from one to five *Atherina* pineals. *Atherina* was chosen as the donor species, because the large size of the organ and its yellow color made it visible under the dissecting microscope. The host *Cyprinodon* were observed from immediately after the effects of anesthesia had worn off, until five or six days after implantation. At no time was there any indication that the implanted pineals had an effect on the pigmentation of the hosts.

#### ***Amieurus nebulosus* (LeSueur)**

The individual used for histological purposes was approximately 10 months old. It was fixed in Maximow's fluid and stained with Heidenhain's azocarmine stain. The anilin blue stained collagenous tissue strongly, and the basement membrane on which the pineal cells rest stood out very clearly.

The stalk is very long and thin and reaches so far anteriorly from the pedicle that the vesicle has its posterior end about midway of the forebrain, and the main body of the vesicle lies over the olfactory nerves. At this age, there is no bone or cartilage between the pineal and the epidermis. A thin band of heavy collagenous tissue lies immediately over the organ, separated from the collagenous tissue on which the skin epithelium rests by a wide band of loose areolar tissue. Both dermis and epidermis are heavily invested with melanophores.

The vesicle is a simple sac, without convoluted walls, and extremely long in an anteroposterior direction. Two types of cells are obvious. In one, the open, vesicular nucleus

typically shows a brighter red-stained nucleolus which may be anywhere within the nucleus, in the center or next to the nuclear membrane. The second type of cell is less numerous, the nucleus is more highly chromatic, and it may or may not show a nucleolus. Cell boundaries cannot be distinguished. The cytoplasm takes a pinkish gray stain. The lumen of the vesicle is fairly well filled with cellular debris, and no exact structures can be defined.

The stalk contains a lumen which widens at the pedicle. It is lined by the same kind of cells seen in the vesicle and also contains parts of cellular elements. No communication with the third ventricle was seen in sections.

#### *Gymnosarda pelamis* (Linnaeus)

The bonito has a window-like, clear space on the top of the head which cannot be seen in the live fish. When the skin is stripped off and held up to the light, the clear space can be seen exactly as Rivas (1953) has described for the tuna. There are no scales over the pineal region, and the skin is adherent to the skull. Over most of the head, the skin epithelium rests on a heavy layer of collagenous fibers; under the collagenous layer lies the pigmented layer, which in turn rests upon a layer of adipose tissue. The adipose tissue lies between collagenous and muscle layers, the muscle being attached to the bony skull. Immediately above the pineal organ, however, this arrangement is modified in the following manner: the area of collagenous tissue is considerably widened and rests directly upon the hyalin cartilage of the chondrocranium immediately beneath which is the large sac of the pineal vesicle. Skull bone, musculature, fat, and pigment layers are omitted in this area, and light is permitted to strike upon the pineal body through translucent cartilage and collagen. There appears to be no mechanism for covering the pineal area with melanin or any other material in order to exclude the light. These features are seen in plate 2, figure 3, which is a photomicrograph of a longitudinal section showing the area of transition from the opaque covering of most of the head to that of the pineal area. Quantities of adipose tissue are characteristic of both this species and the tuna. In the photograph the large fat cells can be seen

within the interstices of the bone of the skull and beneath the skull bone lying between it and the brain. Immediately beneath the pineal vesicle is seen a mass of granular acellular material which fills in between the ventral surface of the pineal vesicle and the brain, which in this case lies quite far beneath the pineal vesicle.

The pineal organ has an extremely long stalk which reaches from the short pedicle to the roof of the cranial case and which is closely associated with the posterior wall of the dorsal sac. The stalk is attached to the sac-like vesicle at its extreme anterior tip; thus the main body of the pineal lies posterior to the stalk, contrary to the situation in most other species.

The vesicle is a broad, sac-like organ, spreading out underneath the whole clear area. The gland appears to be a very simple one; the walls are not convoluted, and the large lumen is therefore undivided. The inner surface of the wall, however, is tufted or provided with short villi. Processes from the cells on the sides of the villi intermingle, or extend into the lumen. There is some scattered debris in the center of the lumen, which was originally cellular in nature. Beneath the pineal cells the walls are richly endowed with capillaries.

The relationship of the various tissues is seen in text figure 1, which is a composite drawing of several sections. The figure represents a sagittal section through the brain very nearly in the midline. Brain tissue is indicated by the stippled area. Cartilage is filled in with solid black, in this area seen in part of the chondrocranium anteriorly and over the pineal area where it connects the bone on either side of the foramen. Collagenous tissue is indicated by closely spaced horizontal lines and muscle by widely spaced horizontal lines. Bone is indicated by curved, uneven lines. The junction of the stalk with the brain itself is not shown in the diagram. Any communication between the lumen of the stalk and the third ventricle was not identified. However, this could easily be concealed in the sections, as the large size of the tissue necessitated the cutting of sections 15 to 20 $\mu$  thick. The pineal vesicle is seen to resemble a pennant flowing posteriorly from its junction with the stalk. It is situated immediately

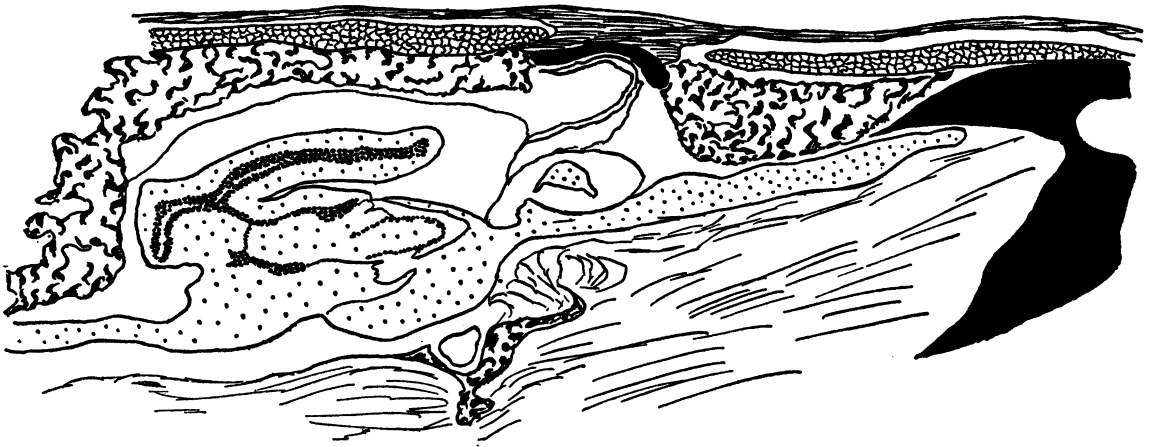


FIG. 1. Diagrammatic representation of the brain of *Gymnosarda* and *Thunnus*, showing the relationship of the pineal organ to the parietal foramen of the skull. See text for explanation.

beneath the cartilage which fills in the foramen of the skull. The stalk is extremely delicate and not nearly so wide as it appears in the figure. True size relationships could not be shown, as the lines representing the walls of the stalk would have been too close together for proper reproduction. All these morphological details apply equally well to the tuna, except that the brain is buried deeper and thus the pineal stalk is longer.

#### ***Thunnus albacares* (Bonnaterre)**

An elaborate morphological arrangement is present in the tuna which permits light to reach not only the pineal but a large part of the brain as well, including all the forebrain and some of the olfactory nerves. Rivas (1953) has already described the anatomy of this region but was unable to find a pineal organ in gross dissection. Histological preparations of a small, 3-pound specimen show the pineal to be present. It has a large, sac-like vesicle; the walls of the vesicle are thin. The same two kinds of cells are found, one smaller with more hyperchromatic nuclei than the other. Short, blunt villi are found projecting into the lumen, and cell processes projecting into the lumen are also plentiful. The lumen itself is very wide and empty except for the cell processes and some cellular debris. There is no sharp line of demarcation between the stalk and the vesicle, and a lumen was seen traversing a considerable distance down the stalk towards the pedicle. The thin connective-tissue capsule of both

vesicle and stalk is plentifully supplied with blood vessels. The pedicle is short and broad.

The epithelium covering the head of the fish is very thin, and there are no epidermal melanophores. A more or less spongy layer of connective tissue lies directly beneath the epithelium, and below this is found a wide compact layer of heavy collagenous tissue. Melanophores are present in this layer and seem to be associated with the blood vessels in it. The dark band of closely packed melanophores which gives the fish its dark color lies beneath this collagenous layer. A narrow band of loose areolar tissue intervenes between the melanic layer and the musculature. The musculature in turn is closely applied to the bony skull. This arrangement of tissues is abruptly modified over the region of the pineal. The collagenous layer is considerably deepened, and, although blood vessels appear in it, their walls are devoid of accompanying melanophores. The collagenous layer is directly applied to a plate of hyalin cartilage which fills in the foramen that is such a prominent feature of the tuna skull. The layers of melanophores, areolar tissue, and muscle are completely lacking. This condition accounts for the transparency of the "pineal window" described by Rivas (1953). The large vesicle of the pineal body lies directly below the cartilaginous plate.

The stalk is extremely long and fragile and is attached to the anterior portion of the vesicle in the same manner as in the bonito. The stalk is supported throughout its lower

half by the pallium of the brain to which it is closely applied. The upper half of the stalk runs through a large core of extremely loose transparent tissue. This tissue reaches from the ventral surface of the pineal vesicle down to the surface of the brain. It is finely granular and acellular in character and shrinks badly during the technical histological procedures. It is occasionally traversed by a capillary and appears to be divided into irregular masses by septa made up of single cell layers; these cells are extremely long and thin, resembling endothelium. The pallium of the brain extends dorsally into this material oriented towards the origin of the pineal stalk from the vesicle.

***Nomeus gronovii* (Gmelin)**

There are no scales over the pineal area, and the whole head is darkly pigmented. Melanophores are numerous in both the thick epidermis and dermis. Immediately beneath the dermis lies a layer of musculature. Beneath that lies the skull bone, and beneath that, in turn, a rather wide band of the cartilage of the chondrocranium. The skull is present but very thin over the pineal area. The stalk is long, inclines anteriorly from the pedicle, and at its base the lumen is in communication with the third ventricle. The vesicle is rather small and has a wide lumen which contains a considerable quantity of cellular debris.

Plate 3, figure 4, is a photomicrograph of a sagittal section through the pineal organ in *Nomeus*. The opening at the lower left is not that of the pineal stalk but the dorsal sac, which in this species is very narrow but long and reaches dorsally to the ventral surface of the chondrocranium closely associated with the pineal stalk.

***Bathygobius soporator* (Cuvier and Valenciennes)**

No scales are present on the head of this species. The dermis covering the head is invested heavily with leucophores, melanophores, and xanthophores in various combinations in chromatosomes. An area just posterior to the eyes has fewer chromatophores, and an opaque white region can be seen deep within the head. However, after dissection this white area is seen to be not over the pineal or any part of the brain, with

the possible exception of the olfactory lobes. A low occipital crest rises from the skull immediately posterior to the eye sockets. The crest supports a fairly thin layer of muscle. The meninges are heavily invested with chromatosomes, combinations of melanophores and leucophores, xanthophores, and leucophores, or combinations of all three types of cells. A tiny, translucent area devoid of chromatophores lies over the pineal area, under the occipital crest.

Formalin-fixed sections stained with hematoxylin and eosin show the pineal vesicle to be a small, simple sac with a wall that is not at all convoluted. Blood vessels are quite prominent in the capsule. The organ is buried deep beneath the surface and is covered by cartilage, heavy bone, musculature, and thick epithelium.

***Opsanus* sp.**

This species is also without scales. The skin is thick and contains numerous mucous glands. It also carries an abundance of chromatophores, coarse melanophores with blunt dendritic processes, xanthophores, leucophores, and iridophores. No conspicuous occipital crest supports the musculature of the head region as is seen in other forms. Muscles are inserted just posterior to the eyes, and the skin is loosely applied to the skull bones anterior to this insertion. The blood vessels of the fairly heavy musculature are surrounded with melanophores. The bone of the skull anterior to the insertion of the musculature is opaque white, but under the musculature it is less opaque, and the melanophores of the meninges can be seen through it when the muscles are dissected away. The skull bone is thick and spongy, but, when it is removed, the meninges can be seen to bear leucophores as well as melanophores. The pineal area lies deeply under the skull, and no area of the meninges above it is devoid of chromatophores.

***Haemulon sciurus* (Shaw)**

The brain of the grunt is buried deep within the head. The top of the head is covered with melanophores, which, when in a dispersed condition under anesthesia, can provide an almost completely black covering except for the places marked by sensory pores. The stripes are obliterated under these

conditions. What xanthophores and iridophores are present lie beneath the melanophores and appear only when the granules of the melanophores are concentrated. The dermis is very thick, and the scales are spiny. Heavy muscle layers lie over the top of the skull; the most dorsal layer of muscle is inserted anterior to the eyes. The occipital crest supporting the heavy musculature arises from the skull at about the level of the eyes. The skull over the top of the brain is thick and spongy. Beneath the skull, between it and the meninges, is a wide layer of fat. No chromatophores were seen in the meninges. The optic lobes set below the forebrain and cerebellum; thus the pineal area is on a level with the posterior edge of the eye.

The pineal organ lies deep beneath scales, dermis, musculature, and bony and cartilaginous skulls. The stalk inclines anteriorly, and most of the vesicle lies over the posterior part of the forebrain. The vesicle has a wide lumen; the walls are infolded, forming stout villi which protrude into the lumen but do not subdivide it. The cells are small; there are two kinds of nuclei, one more hyperchromatic than the other, but both are round. The connective-tissue capsule is richly supplied with capillaries and larger venules. Cell processes are not conspicuous; the lumen is clear. Plate 4, figure 1, is a dorsal view of the head of *Haemulon*, showing no modification that would give a clue as to the position of the pineal area. Plate 4, figure 2, is an oblique transverse section showing the small size of the vesicle in relation to surrounding structures.

***Thalassoma bifasciatum* (Bloch)**

This species is characterized by a marked sexual dimorphism. No scales are found over the top of the head in either male or female. In the male, sensory pits make the only translucent areas in an otherwise solidly pigmented dermis. The color over the head as well as over the entire anterior region of the fish is a bright turquoise blue. This effect is gained by xanthophores, melanophores, and iridophores of minute size. The occipital crest supporting the musculature rises from the skull just anterior to the pineal area. The dermis is closely applied to the skull over the

region of the frontal lobes, but the pineal region is covered not only by the heavily pigmented skin but also by musculature and a fairly thick skull. The bone of the skull is transparent, and, when the muscles are dissected away, the dura can be seen through it, sparsely invested with iridophores, xanthophores, and small melanophores. An occasional large-sized melanophore is seen. The pigmentation of the meninges covers the pineal area, leaving no translucent area.

In the female, the bright blue coloration is lacking. Again sensory pits form the only translucent areas in the pigmentation of the head. The coloration of the head is either yellow or black, depending on the condition of dispersion or concentration in the melanophores and xanthophores of the skin. Iridophores are also present but not in such quantities as in the male. The male has more iridophores and fewer xanthophores; the female has more xanthophores and fewer iridophores. This condition results in permitting more light to pass through the dermis in the female. The morphology of the skull and musculature is the same as in the male, including the meninges the chromatophores of which cover the whole brain, including the pineal area.

***Irideo bivitatta* (Bloch)**

The pineal area is covered by scales, a heavy layer of musculature, bony skull, and chondrocranium. The vesicle is a small one, lying immediately over the junction of frontal and optic lobes. The walls are convoluted, dividing the lumen into many spaces. The stalk is very broad, and its walls also are convoluted, making considerably more surface area of pineal cells than is usually seen in this part of the pineal complex. The lumen extends down to the pedicle, but no communication with the third ventricle could be found. The pedicle is short. The same three types of cells are noted: supporting cells, and two epithelial components, one with smaller and more hyperchromatic nuclei than the other. Cellular elements protruding into the lumen are short and stain a faint bluish purple with Heidenhain's azan connective-tissue stain. The fluid within the dorsal sac, on the contrary, stains a clear, bright blue.



**Spheroides spangler** (Bloch)

The skin of this puffer is not particularly heavily pigmented. The skull dorsolaterally is composed of heavy, spongy bone, but over the pineal area the bone is reduced to a very thin layer immediately above the chondrocranium. Between the skull and the dermis are two heavy layers of connective tissue. The brain is an extremely interesting one from the point of view of morphology. The photograph of plate 4, figure 3, is given to show the extraordinary shape of the optic lobes as well as the location of the small pineal vesicle. The pineal stalk is thick, with a wide lumen in which are found many cell processes and a considerable quantity of cellular debris (pl. 4, fig. 4). The stalk lumen is lined with pineal cells all the way to its base. The pedicle is extremely short, and there is definitely a very narrow opening in communication with the third ventricle. From its base upward, the stalk continues to widen and ends without constriction in the wide vesicle. The stalk is short, and the vesicle is situated well below the level of the top of the optic lobes (pl. 4, fig. 3). The walls of the vesicle are not particularly convoluted, although a few blunt villi protrude into the lumen.

Three types of cells are noted here; all are somewhat larger than those seen heretofore in other species. One type has large, vesicular nuclei which are either round or oval depending on the plane of section. The second type has the smaller, more hyperchromatic nuclei; these two types occupy the basal layer of cells. A second layer of cells, distal to these, contains the third type of cell. In these the nucleus is small, and the chromatin does not stain heavily. These appear to be the cells that provide the processes streaming into the lumen. Often the whole cell, including the nucleus, protrudes into the lumen, so that the general impression is that the cells themselves are being extruded. This would account for the cellular debris found in the lumen of the vesicle and stalk.

The pineal organs of this species probably would offer better material for cytological study than those more highly differentiated ones used for the present report, because of the larger size of the cells, the uncomplicated

walls, and the short stalk. This last would make possible dissection of the brain with the pineal intact, protected by the higher optic lobes, and the situation of having the long stalk break and leave the vesicle in the skull would be eliminated.

**Histrio histrio** (Linnaeus)

The morphology of the pineal region in this species was studied from sagittal and transverse sections of two juvenile individuals. In sagittal section, it can be seen that almost the whole brain from the anterior end of the forebrain back to the medullary region lies in that part of the head between the first and second great dorsal spines. The skin is without scales. The pineal lies under the cartilaginous chondrocranium, thin skull bone, a layer of loose areolar tissue, a band of collagenous tissue, and skin. Melanophores are distributed in the dermis over the top of the head irrespective of the position of the pineal organ. The body musculature is inserted posteriorly, about midway of the optic lobes. The vesicle of the pineal reaches up to the chondrocranium in one area, but most of the organ lies beneath the level of the optic lobes. This can be seen in transverse section in plate 5, figure 1.

The vesicle is fairly small, with a wide band of pineal cells the processes of which nearly fill the lumen. These cells also line the very short stalk. In the short pedicle, where the lumen passes down just posterior to the habenular commissure, the lining seems to be of a simple epithelial type. Fibers are scarce. The lumen of the stalk has a narrow opening to the lumen of the third ventricle. No dorsal sac could be identified, but a large velum transversum is present. This can be seen in the photograph of plate 5, figure 1, beneath the habenular ganglia. The capsule of the pineal is richly vascularized and contains melanophores. The presence of melanophores in the capsule of the pineal organ itself was not noted in any species described up to this point.

**Hippocampus reidi** (Ginsburg)

The head of the sea horse is covered with short, pointed, bony plates. The outside epithelium is thin, and immediately beneath

it is a dense layer of melanophores. A wide band of collagenous connective tissue lies between the dermis and the very thin, compact bone of the skull. Beneath the skull lies another layer of collagenous tissue. No part of a chondrocranium is present over the pineal area. The space between the collagenous layer and the surface of the brain is filled with an acellular, coagulated precipitate similar to that seen in the tuna and bonito. The pineal organ is partly supported by this material, and blood vessels from the velum transversum run beside the pineal capsule, attached to it, and rise from the top of the pineal organ to the most dorsal part of the cranial space. Here they send branches both anteriorly and posteriorly. A few melanophores are associated with these blood vessels, both within and outside the pineal capsule.

In this species, also, there is no dorsal sac, but a well-developed velum transversum. The pineal organ is an extremely simple one. A narrow opening connects the lumen with the third ventricle. There is so little differentiation in this organ that the three parts ordinarily described are not seen. The organ is short, wide, and terminates bluntly without spreading out dorsally into any kind of vesicle. It does not extend dorsally above the level of the lobes of the forebrain. The lumen is wide and filled with cell processes. There is no convolution of the walls. The whole organ occupies the place where the stalk alone is found in other species. These features can be seen in the photograph of plate 5, figure 2.

The thin connective-tissue capsule is ex-

tremely well vascularized. The cells lining the lumen present at least three and possibly four different types of nuclei. In the specimen sectioned, many of the most predominant cells are vacuolated in the part of the cell next to the basement membrane. The nuclei thus appear parted from the bases of the cells by this row of vacuolations. Small hyalin bodies are seen in abundance in the lumen, which are parts of dead cells.

#### *Monacanthus ciliatus* (Mitchill)

In this species, the specialization of the body form is such that the morphological position of the organs differs from that in most other teleosts. In transverse section the area of the brain containing the pineal is at the same level as the kidney, liver, and spleen. The optic nerves reach anteriorly to meet the eyes; the pineal region is extraordinarily reduced, as is the dorsal sac (pl. 5, figs. 3, 4). The brain is buried deep beneath the skull and a large quantity of muscle that supports the great dorsal spine. In addition, the pineal vesicle does not reach dorsally above the surface of the brain but lies at least two-thirds of the way down between the two lobes of the forebrain. The photograph of plate 5, figure 3, is of a slightly oblique transverse section showing the position of the pineal vesicle, which is very simple in structure, with a wide lumen and unconvoluted wall. Cell processes and cellular debris fill the lumen. The stalk is short, and the pedicle is very small.

### CYTOLOGY OF THE PINEAL ORGAN

Many years have elapsed since any investigators have been seriously interested in the teleost pineal, and it seemed possible that the application of modern histochemical methods might resolve some of the questions which still remain unanswered as to the structure and function of this organ. While its form varies widely among different species, it is obviously neither vestigial nor rudimentary, as it exerts a great influence over the behavior of many teleosts.

Some ingenious hypotheses concerning the function of the pineal cells have been advanced by various investigators working in the field of histology. These have been re-

viewed for the fishes by Friedrich-Freksa (1932). The main difficulty arises from attempts to reconcile the obviously glandular function of the organ in higher vertebrates with what has seemed to be an almost equally obvious sensory function in lower vertebrates. Von Frisch (1911a, 1911b) definitely established a light-perceptive function for the pineal area in *Phoxinus laevis*. Because the reaction of melanin dispersion in chromatophores in response to light stimulation was not abolished in this species after pineal extirpation, von Frisch concluded that the sensory perception was not confined to the organ alone but was included in adjacent

brain areas, probably in the epithelium lining the ventricle of the diencephalon.

All the teleost pineals studied for the present report were found to have the same basic cytological structure. Differences between the species are confined only to sizes and shapes of the pineal cells, and to the gross morphology, that is, differences in the length of the stalk, the size of the vesicle, and the convolution of the walls. All teleost pineal organs are surrounded by a thin connective-tissue capsule in which are capillaries and larger blood vessels. More or less fibrous tissue lies between the outer membrane and the basement membrane upon which the cells of the pineal parenchyma rest. The fibrous layer is composed of reticular and nerve fibers. There are at least two types of cells in the pineal parenchyma, if one may judge from differences in nuclei. One of these has small, hyperchromatic nuclei, and the other has larger, more vesicular nuclei which appear to belong to the functional pineal cells. Cell boundaries are extremely indistinct. In some species the differences between the two types of cells is very obvious; in others it is not so clear. A third type of nucleus which has the appearance of a connective-tissue cell probably belongs to supporting cells. Occasionally four types of cells have been recorded in the descriptions given above. In part this number probably results from different planes of section through the cells with the vesicular nuclei, giving a round appearance when cut transversely and an oval one when cut sagittally. Another probability to be considered is that an apocrine secretion is formed in this organ, that the cells that provide the secretion show nuclei in various stages of pycnosis, and that these are responsible for the seemingly several varieties of cells. Ganglion cells have been described by Tretjakoff (1915) for *Petromyzon* and by Holmgren (1920) for *Squalus*, and the presence of these cells has been confirmed in *Atherina* pineals in the present observations.

Cell processes are described by many observers as extending from the pineal cells into the lumen. It is extremely doubtful whether these are true cell processes, that is, that they are a permanent part of the cell, as are cilia or brush borders or sensory hairs. The appearance of these so-called processes varies not only with the fixative but also with

the stain. Plate 6, figure 1, is a photomicrograph of an *Astyanax* pineal fixed in Bouin's fluid and stained with Masson's trichrome. Here small cell processes seem to be obvious, extending from the distal parts of the cells into the lumen. Plate 6, figure 4, is a Bouin-fixed pineal of *Cyprinodon* stained with Sokol's modification of Halmi's stain. Plate 7, figure 4, is a photograph of an *Astyanax* pineal, also Bouin-fixed and stained with Gomori's aldehyde-fuchsin. Although the last two pineals mentioned were fixed with the identical agent, the contents of the lumina appear to be cellular debris rather than cellular processes. Cell processes are noted in the photograph of plate 6, figure 2, which is an *Astyanax* pineal fixed in Maximow's fluid and stained with Heidenhain's azocarmine. In other words, if one stains for the cellular structure of the pineal, the cells will appear to bear processes; if one stains for a positive reaction of the material in the lumen, extrusion products and cellular debris become the obvious features.

In the *Astyanax* pineal fixed with Maximow's fluid and stained with Heidenhain's azocarmine (pl. 6, fig. 2), the collagenous fibers and the basement membrane are sharply differentiated from the rest of the tissue by their affinity for the anilin blue. Two kinds of epithelial cells are distinguished. One type has a large, round, vesicular nucleus and is less numerous than the second type which has a more oval hyperchromatic nucleus stained a deep red by this method. Cytoplasm of both types of cells is scanty and takes very little color in this stain, appearing only grayish. Processes which are also nearly colorless extend into the lumen, and scattered along this ragged edge are hyalin bodies that resemble degenerating nuclei palely stained with Orange G. These processes represent bits of cell membranes and cytoplasmic debris of degenerating cells that have been sloughed off into the lumen. In some places the whole inner layer appears to be sloughing off. The *Amieurus* pineal subjected to the same technique shows a much greater quantity of debris in the lumen with a corresponding increase in orange-stained hyalin material.

The figures of plates 6 and 7 demonstrate the difficulty in identifying cell processes or extrusion products and indeed of separating

one cell from another. However, among all the different species studied, no evidence was seen of the elaborate cell types described by Friedrich-Freksa (1932), although his results were obtained by the use of Heidenhain's stain.

Several stains were employed with negative results. No mucin was found in any pineal after its having been stained with Mayer's mucicarmine or diluted toluidine blue. Gallocyanin, used after formalin fixation, showed a complete lack of cells containing Nissl substance. Sections stained for neurosecretion with Gomori's chrome-alum-hematoxylin and phloxin were also negative in the pineal, although the neurohypophysis present on some of the same sections showed strong, positively stained areas.

Some positive results were obtained for lipids after staining with Sudan Black B and Baker's acid hematein for phospholipids. These positive results were probably due to the staining of mitochondria in the cells.

The impression of an inner degenerating layer of cells is strengthened by a study of the pineal of several species stained with Sokol's modification of Halmi's paraldehyde-fuchsin technique. Plate 6, figure 4, is a section of *Cyprinodon* pineal stained by this method. Nuclei are unstained but are distinguishable under the oil immersion lens when small changes in focus are made. Collagenous fibers are stained light green, and the pineal cells are nearly colorless. In contrast much of the debris within the lumen stains deep purple with paraldehyde-fuchsin. The debris seems to contain two types of degenerating cells. One is probably derived from the epithelial cell with the large vesicular nucleus; the degenerating nucleus is hyalin and pale green in color. The second type has an unstained degenerating nucleus, but the cytoplasm is deeply stained and homogeneous. The second type of cells gives the color to the lumen when this stain is used.

These features are not quite so clear in tissue stained with Gomori's aldehyde-fuchsin without previous oxidation. With this stain the cell membranes of the degenerating cells in the lumen stain most intensely regardless of which type of cell they originated from (pl. 7, fig. 4).

If there is a secretion in these teleosts

pineals, it is an apocrine one, as already suggested by Grunewald-Lowenstein (1956), who described the histological details of the pineal organ of *Astyanax mexicanus*. She noted the presence of disintegrating cells within the lumen and was led to believe that the cells with the large vesicular nuclei actively migrated into the lumen. After using glycogen-staining techniques she concluded that an apocrine secretion was in process which released glycogen into the lumen. The outlet for such a secretion must therefore be through the communication with the third ventricle, the secretion becoming part of the cerebrospinal fluid. In teleosts the pineal organ does not appear to be strictly endocrine in nature. Blood vessels are confined to the connective-tissue capsule at the bases of the pineal cells. No capillaries penetrate the pineal parenchyma.

Positive results were obtained for glycogen with the Bauer-Feulgen stain as well as with PAS. Decalcified sections of *Astyanax* pineal regions were stained in PAS, and the results showed a considerable amount of glycogen within the cells as well as in the lumen. Plate 7, figure 1, shows the organ stained with PAS, and figure 2 of the same plate shows the same stain after saliva digestion. Unfortunately the color does not lend itself very well to photography, so that the loss of staining reaction in plate 7, figure 2, is not nearly so clear as it is when seen under the microscope. The positive reaction of the capillary wall is still seen to be present after saliva digestion.

Both *Atherina* and *Cypselurus heterurus* (Rafinesque) were chosen for experiments to determine whether light and darkness had an effect on the glycogen content of the pineal organ. *Atherina* was used because of its large organ which is unprotected from sunlight by any special covering. Immature *Cypselurus* was used because of the peculiar reaction of this fish to light. In the immature stage the pineal region is completely exposed, and the animals may be killed at night by the simple procedure of turning a flashlight beam on the top of their heads. As controls, fish living in ordinary daylight conditions were killed, fixed, and stained with PAS. The experimental fishes were placed in a tank in a totally dark room. Two fishes of each species

were sacrificed at two, four, six, and 24 hours after having been placed in the dark. They were killed by being dropped into Bouin's fluid; the heads were removed from the bodies, and the eyes were also removed to insure proper fixation. This technique was carried out in a darkroom that was illuminated only by a photographic red electric light.

The remaining fishes were returned to the bright light of the laboratory at the end of 24 hours. Two fishes of each species were sacrificed immediately, and two fishes of each species were killed at two, four, six, and 24 hours after having been removed from darkness.

The results in *Cypselurus* were inconclusive. In sections from the controls, a faint, positive, PAS reaction was shown in the lumen, and in one organ there was some faint staining in the cells. There was no change in the pineals of all the fishes killed in darkness, nor was there any difference shown in the organs after a return to light.

Grunewald-Lowenstein (1956) has demonstrated in *Astyanax* that constant exposure to light or darkness has an effect on the glycogen content of the pineal organ but only after prolonged exposures. The immediate and violent reaction of the young of *Cypselurus* to abrupt application of light at night must be associated with some shock mediated through the nervous system, either through the eyes, or the pineal organ, or the brain itself. Considerable areas of the brain are exposed in the young stages.

In *Atherina*, the positively stained material occupied a different area from that seen in sections stained by the Bauer-Feulgen technique. This is not significant, because the PAS technique was not carried out on material fixed in ice-cold solutions, and the glycogen present probably was not in its original position. In the control PAS sections, a staining gradient was observed, with the faintest stain in the pineal cells near the roof of the skull and the deepest stain down near the stalk. The stain was seen mostly in the cell processes in the lumen, although fine strands that assumed odd shapes were seen within the cells. These somewhat resembled a Golgi network, except that they were not oriented to the nucleus in any way. There was

no change in this picture in animals kept in darkness until the end of six hours. Sections of one of the two fishes killed at this time showed the pineal to be covered with fairly large, dark red blotches. This material seemed to have no relation to the cells or the lumen but was scattered indiscriminately throughout the organ. The blotches were definitely not due to any precipitation of stain; they were confined to the pineal and were completely absent from adjacent areas of brain tissue and third ventricle. Sections from fishes kept 24 hours in darkness showed similar blotches less intensely stained. The centers of many were colorless so that they appeared as positively stained rings. In the two fishes killed immediately after return to light, one showed an abundance of similar deeply stained material and in the other the blotches were identified but had retained none of the stain. Sections of the pineal organs of the fishes killed during the 24-hour period after removal from darkness showed a more or less gradual return to the normal picture, with positively stained material confined to small spots in the cells and to the cell processes seen in the lumen.

The presence of glycogen in pineal cells indicates only that the material is being stored there. From the results described in the foregoing experiment, the pineal body can store glycogen during hours of darkness. During the daylight hours, increase in cellular metabolism and consequent use of the glycogen could account for its near disappearance. This means only that light stimulates the pineal to greater metabolic activity. Grunewald-Lowenstein's suggestion (1956) that the pineal cells secrete glycogen into the lumen in an apocrine fashion is confirmed in the present report. The apocrine secretion containing glycogen and glycoprotein is delivered to the cerebrospinal fluid through the communication with the third ventricle. Whether the pineal secretion contains a hormone or not cannot be determined by the methods used in the present report.

The results of the use of Bodian's silver-protargol stain for nerve fibers can be seen in plate 7, figure 5. Figure 6 of the same plate is a detail of the lower right corner of figure 5. These photographs show nerve fibers in the convolutions of the walls of the pineal vesicle



in *Atherina stipes*. Plate 6, figure 3, is a photograph of the *Atherina* pineal, after use of Bielschowsky's method, showing a free nerve ending. Fine nerve fibers are plentiful in the capsule, and small branches of these enter the layers of pineal cells to end in small roundish knobs. Because of the poor fixation of this technique, cell boundaries are nearly impossible to determine, and whatever relationship these free nerve endings have to the pineal cells cannot be established. Many of the nuclei show heavy blackened networks, but in no case were these seen to be connected to fibers, and that these are artifacts of precipitated silver is shown by their presence in the nuclei of some erythrocytes also. Similar nerve endings were seen in *Strongylura* pineals stained by this method, but no further cytological details could be observed.

Bielschowsky's method demonstrated free

nerve endings in *Tylosurus raphidoma* and *Atherina stipes*. The endings were frequently, but not always, closely associated with nuclei of pineal cells. Where the tissue was properly stained the nerve endings were seen to be fairly numerous. Larger nerve fibers were associated with the blood vessels in the pineal capsule, and some free nerve endings could be observed along the blood-vessel walls.

In transverse sections of the *Atherina* pineal stained with Bodian's method, an aggregation of small nerve cells was noted at the base of the vesicle just where it narrows to form the stalk (pl. 7, fig. 3). These are small cells with short dendrites and longer axons. The terminations of the axons could not be determined, but they run down the walls of the stalk and enter the habenular commissure.

#### EFFECTS OF PINEALECTOMY IN *ASTYANAX MEXICANUS*

Pflugfelder (1953, 1954) ascribes a pineal-pituitary relationship to the teleosts. He has described a distortion of the vertebral column, hypertrophy and hyperplasia of the thyroid, and changes in the hypophysis as a result of pinealectomy. He does not elaborate on the subject of hypophysial changes. These endocrine changes were reported (1953) as a result of pinealectomy in one-day-old *Lebistes*. His second paper more or less furnishes a control for the first, as in this one (1954) he made different kinds of brain lesions, and only in the cases in which the pineal was destroyed did the described endocrine changes occur. The technical difficulties involved in operating on such small fish must have been considerable. For purposes of confirming Pflugfelder's results, an experiment in pinealectomy was conducted for the present report. No such results as those described for *Lebistes* were obtained in *Astyanax*. A description of the experiment and its results follows.

Specimens of *Astyanax mexicanus* were used for the experiment. At the time of operation they were two months old, and none was sexually mature. Twelve fish were used for controls, and 22 were operated on in an attempt to remove the pineal. Standard lengths of the fish were measured while the

fish were under anesthesia, in as much as the growth rate of the fishes could be interfered with if there were any destructive effects of pinealectomy on the hypophysis.

The fish were anesthetized in 1 per cent urethane. The pinealectomies were performed by lifting a flap of the skull with the skin attached, scraping the inside of the skull with a sharp scalpel, and sucking with a pipette at the area between the optic and frontal lobes of the brain where the pineal stalk emerges. The flap of the skull was then replaced, and the fish were kept in physiological saline for about a week. After that time the wounds had healed, and the fish were returned to fresh water. The use of physiological saline prevented osmoregulatory disturbances which would occur if fresh water were allowed access to the open wounds. Among the 22 fish so operated on, there was only one fatality. At this early age the bones of the skull are not yet very hard, and little cracking occurred from the bending of the skull back upon itself. However, lesions in the forebrain were unavoidable; consequently forebrain lesions were made in the controls. The points of watchmaker forceps were inserted through the skull over the forebrain and into the frontal lobes. For these controls a flap of

skull could not be lifted nor could the skull be cracked lest the delicate pineal stalk be broken.

In *Astyanax* the pineal gland cannot be seen under the dissecting microscope when the skull is opened. The reason for attempting this method of pinealectomy lies in the fact that it is extremely difficult to dissect a fish brain with the pineal intact. The usual occurrence is that the stalk breaks and leaves the vesicle adhering to the inner surface of the skull. The operation therefore consisted in an attempt to remove something that could not be seen.

The fish were allowed to live for 14 months postoperatively. They were then tested in a gradient trough in sets of four as described by Breder and Rasquin (1947). After the test they were blinded by section of the optic nerve under anesthesia and tested again in the gradient trough on the following day. The fish were then killed by decapitation and fixed in Bouin's fluid. The heads, thyroid regions, and gonads were sectioned and stained with hematoxylin and eosin or Masson's trichrome connective-tissue stain. The sections were studied in order to check the presence or absence of the pineal and to discover the conditions of pituitary, pineal, and gonad. At the time of sacrifice, the standard lengths of control and experimental fishes were measured.

Study of the sections showed that the pinealectomies were not complete in many cases. Of the 21 fishes surviving the pinealectomy, two died after having been blinded, and one was not sectioned. Eight of the fish showed no trace of pineal vesicle or stalk. Five showed the whole gland and stalk to be present, while seven showed the presence either of the stalk alone or the stalk plus some of the vesicle in a cystic condition. These last were considered to have functional tissue present, because the non-cystic parts of the vesicle showed cellular constituents to be normal histologically. When the stalk alone was left, it did not degenerate, although the cells seemed to have lost a certain amount of cytoplasm, and their orientation towards a lumen was disrupted.

The photomicrographs of plate 8 are devoted to this experiment. Figure 1 of that plate shows the condition in fish no. 6 in

which the whole gland remained intact save for a section of the stalk which probably is an artifact that occurred during the histological technique. Figure 2 shows the complete absence of the organ in fish no. 10. Figure 3 shows the cystic condition of the vesicle in fish no. 18, and figure 4 shows the remnant of the stalk in fish no. 1. In the last figure the stalk cells are in the center of the tissue clump; the cells at the bottom are those of the dorsal sac.

Unfortunately, fishes with these different conditions were scattered throughout the sets used for testing responses to light, as there was no way of determining what their condition was before they were sectioned. None of the live fishes exhibited the "kypholordosis" or distortion of the vertebral column described by Pflugfelder (1953). Outwardly the fish all appeared the same, that is, normal. They are listed in table 1 according to the sets used in the gradient trough, with results of the tests, the standard lengths, and operated condition of each fish.

None of the changes in endocrine glands described by Pflugfelder (1954) for *Lebistes* was seen in pinealectomized *Astyanax*. The histology of hypophysis and thyroid as well as gonads was indistinguishable from that of the controls and did not appear abnormal in any way. There was no significant difference in growth rates of the fishes. At the time of operation, the 12 fishes used for controls varied from 19 to 33 mm. in standard length, with a mean of 22.3 mm. The standard length of the fishes operated on for pineal removal ranged from 17 to 33 mm., with a mean of 22.0 mm. At the time of killing, the control group ranged from 40 to 51 mm., with a mean of 45.8 mm., and the experimental group from 37 to 53 mm., with a mean of 43.7 mm. The slight difference in the mean here might be thought to be the result of keeping 21 fishes in one tank and 12 in the other. However, of the 21 fishes operated on from the single tank, the mean standard length of the completely pinealectomized group was 44.75 mm., of the partially pinealectomized group 41.14 mm., and of the group with intact pineals 42.8 mm. These data do not indicate any effect of pineal removal on growth.

Schönherr (1955) found that destruction of

TABLE 1  
RESULTS OF PINEALECTOMY IN *Astyanax mexicanus*

| Set and Fish Nos. | Condition of Pineal           | Light Sensitivity Reading | Light Sensitivity Reading after Blinding |
|-------------------|-------------------------------|---------------------------|--|
| Set 1             |                               | 0.000                     | 1.000                                    |
| 1                 | Stalk only                    |                           |  |
| 2                 | Stalk and cystic vesicle      |                           |  |
| 3                 | Total pinealectomy            |                           |  |
| 4                 | Died after being blinded      |                           |  |
| Set 2             |                               | 0.000                     | 1.100                                    |
| 5                 | Stalk only present            |                           |  |
| 6                 | Whole pineal present          |                           |  |
| 7                 | Whole pineal present          |                           |  |
| 8                 | Stalk only                    |                           |  |
| Set 3             |                               | 0.000                     | 1.570                                    |
| 9                 | Stalk and degenerated vesicle |                           |  |
| 10                | Total pinealectomy            |                           |  |
| 11                | Total pinealectomy            |                           |  |
| 12                | Total pinealectomy            |                           |  |
| Set 4             |                               | 0.400                     | 1.100                                    |
| 13                | Stalk and cystic vesicle      |                           |  |
| 14                | Total pinealectomy            |                           |  |
| 15                | Whole pineal present          |                           |  |
| 16                | Whole pineal present          |                           |  |
| Set 5             |                               | 0.920                     | 0.840                                    |
| 17                | Total pinealectomy            |                           |  |
| 18                | Stalk and cystic vesicle      |                           |  |
| 19                | Whole pineal present          |                           |  |
| 20                | Total pinealectomy            |                           |  |
| Set 6             |                               | 0.006                     | 0.370                                    |
| 21                | Total pinealectomy            |                           |  |
| Set 7             |                               | 2.014                     | 0.770                                    |
| 22                | Control whole gland           |                           |  |
| 23                | Control                       |                           |  |
| 24                | Control                       |                           |  |
| 25                | Control                       |                           |  |
| Set 8             |                               | 0.002                     | 0.856                                    |
| 26                | Control                       |                           |  |
| 27                | Control                       |                           |  |
| 28                | Control                       |                           |  |
| 29                | Control                       |                           |  |
| Set 6             |                               | 0.006                     | 0.874                                    |
| 30                | Control                       |                           |  |
| 31                | Control                       |                           |  |
| 32                | Control                       |                           |  |
| 33                | Control                       |                           |  |

the epiphysis in *Gasterosteus aculeatus* had no effect on nuptial coloration or reproductive behavior.

The results of the test for light sensitivity are given in table 1. In this test, a score of four indicates that all four fishes tested at the

same time were completely light positive in their reactions. A score of zero means that the fishes were all completely light negative in their reactions. A score of two indicates random movement. (See Breder and Rasquin, 1947.) A study of the table reveals that al-

most all groups of fishes increased slightly in positive reactions after they had been blinded. The two exceptions are set 5 of experimental animals and set 7 of controls. The decrease in light positivity after blinding in set 5 is obviously not significant. Why the reactions of set 7 should be so out of line with those of the other groups is not known but was probably the result of the presence of some extrinsic factor not noted at the time of the test. The increase in light positivity after blinding is due to the loss of vision. These fishes ordinarily station themselves under some form of shelter and look out into the lighted area. Without vision this behavior is modified so that they move in and out of shelter more freely. Those groups containing pinealectomized fishes made only a slightly higher score after having been blinded than did the controls. This difference was so slight that it does not appear that pinealectomy had any real effect on light sensitivity.

The cause for the lack of results on photosensitivity in pinealectomized *Astyanax* is probably to be found in the presence of dense pigmentation. When present the pineal is affected by the amount of light impinging on it (Breder and Rasquin, 1947). The regenerated skin over the tops of the heads of the fish operated on showed no lack of melanin

pigmentation. Not enough light penetrated to the brain areas to make any significant difference between the behavior of pinealectomized and control fishes.

Another possibility is that other brain tissues in the pineal area partake of the reaction to light, as von Frisch (1911a, 1911b) has already postulated for *Phoxinus*, so that pinealectomy alone is not sufficient to bring about behavior changes.

No difference in pigmentation was noted in the pinealectomized or partially pinealectomized fishes from that of the controls. Normal coloration was shown by all groups of fishes.

Hoar (1955) studied the responses in phototaxis in *Oncorhynchus* juveniles after partial destruction of the pineal area. These operated animals cannot be considered as pinealectomized, as the operation consisted of probing the pineal area with a needle, and the photomicrographs of sections of the fish operated on show a considerable quantity of pineal tissue remaining. The experiment, however, was well controlled and demonstrated that the pineal area was sensitive to light. The effect of partial destruction of the pineal on the pigmentation was not very pronounced, the fish operated on being darker than control fish but lighter than blinded fish.

## PART 2. REACTIONS OF THE PIGMENTARY SYSTEM TO HORMONAL STIMULATION

THE INFLUENCE OF various hormones on the pigmentation of teleosts has been studied for the most part by experimentation with fresh-water fishes. Most of these reports indicate that the hormone of the intermediate lobe of the pituitary causes dispersion of pigment granules within the melanophores and that adrenalin causes concentration of pigment granules within the same cells. There is also considerable evidence to show the presence of a melanophore-concentrating hormone in the pituitary which was first described by Hogben (1942). The literature in this field up to 1943 has been thoroughly reviewed by Parker (1948), and literature pertaining to the chromatophore hormones of the fish pituitary has been more recently reviewed by Pickford and Atz (1957). Some investigators, such as Meyer (1931) and Weisel (1950), have noted that this reaction of melanophore dispersion to intermedin is not shown by all fishes. Other pituitary hormones, such as ACTH, have been demonstrated to have melanophore-dispersing activity (Kohler, 1952), and melanogenesis in yellow goldfish has been stimulated by ACTH (Chavin, 1956).

The evaluating of the literature on this subject is difficult because of the lack of standardization in the hormones involved. The hormone preparations that have been used range from fairly well-purified mammalian extracts to crude whole-fish pituitaries of various species. The use of various kinds of teleosts as test animals has shown that all do not react in the same way; responses of teleosts are not nearly so uniform as those of anurans. This is not surprising, in as much as the teleosts have evolved in more different and further directions than anurans. One of the causes of differences in reactions is the nervous control of pigmentation

with which some teleosts can override the hormonal action. Some teleosts have the ability to override the hormonal influence to a marked degree; others are helpless in this regard.

The pigmentary responses that are mediated through visual cues, such as background matching and social behavior, can be eliminated by blinding. In as much as blinding causes the fish to assume a dark phase when kept under the ordinary light conditions of the laboratory, it is useful only for testing materials that will cause concentration of pigment within the melanophores. Although the eyed fish kept in darkness assumes a pale phase, the color is not particularly uniform among a given number of fish of the same species and is subject to regular darkening when lights necessary for observation are turned on.

The present Part 2 of the report describes the pigmentation changes in fresh-water and marine fishes after administration of various pituitary fractions and adrenalin. Thyroxin was also used in one species.

Nine different pituitary preparations were very generously supplied by Dr. Irby Bunding of the Armour Laboratories. Thyroxin was kindly donated by Dr. George S. Reed of the Squibb Institute for Medical Research, and intermedin was supplied by Dr. Henri Choay of the Laboratoire Choay, Paris. Dr. C. M. Breder, Jr., Miss Louise Stoll, and Mrs. Marie Lou Campbell assisted with certain of the experimental procedures. Work with the marine species was carried on at the Lerner Marine Laboratory, Bimini, Bahamas, British West Indies; that with the fresh-water fishes was done in the laboratories of the Department of Fishes and Aquatic Biology, the American Museum of Natural History, New York.

### MATERIALS AND METHODS

Because the effects of adrenalin and intermedin administration in many species of teleosts have never been recorded, they are given here in greater detail than is absolutely

essential for this report. Thirty-five different species of both marine and fresh-water teleosts were used.

The hormones were injected into the ab-

dominal cavity of the fishes. The adrenalin used was epinephrine hydrochloride 1:1000, manufactured either by Parke-Davis and Company or by the Jen-Sal Laboratories. The product of the latter company was intended for veterinary use only. Both contained a small quantity of chlorbutanol. No differences were noted in the reactions of the fishes to the two preparations. The intermedin used was in the form of melanophore-stimulating hormone (MSH lot D216-155-C) obtained from the Armour Laboratories, and intermedin Choay obtained from the Laboratoire Choay, Paris. Dosages and effects of hormone administration are given in the text and in table 2. Dosages of adrenalin were arrived at empirically. At the beginning of the work, the hormone was diluted to 1:10,000 or 1:5000, but this was found to be unnecessary and in some cases ineffective. Thus the more satisfactory procedure of using a standard concentration of 1:1000 and adjusting the quantity of the fluid to the size of the fish was employed. Certain extremely fragile forms could not survive the handling necessary for these simple experimental procedures; these are indicated in the text.

MSH was suspended in 0.6 per cent saline solution, and the dose consisted of approximately 1 milligram per 4 grams of body weight. Doses of this order of magnitude were shown by Chavin (1956) to be sufficiently potent to cause melanin dispersion in gold-

fish. The intermedin obtained from Choay was already dissolved in isotonic saline, some with 4000 units per cubic centimeter and some with 2000 units per cubic centimeter. The salinity of this material was no doubt somewhat higher than 0.6 per cent, as it was probably closer to concentrations found in mammalian blood. Intermedin dosages are indicated in table 2.

In most cases an attempt was made to record pigmentary changes according to the times at which they appeared. Because of the rapidity of some of these, the times given can be considered as only rough approximations and must not be considered as precise quantitative units. They serve to give a general idea of comparative reaction times and the sequence of events between species. These details, together with other data, are given under the headings for the various forms, which are arranged in the conventional taxonomic order.

In addition a separate experiment was performed on *Astyanax mexicanus* and *Bathygobius soporator*, the details of which are given under the proper heading. For this purpose, nine different pituitary preparations were generously supplied by Dr. Irby Bundling of the Armour Laboratories, and the thyroxin was kindly donated by Dr. George S. Reed of the Squibb Institute for Medical Research.

## RESPONSES TO HORMONE INJECTIONS

### ORDER ISOSPONDYLI

#### FAMILY CLUPEIDAE

##### *Harengula macrophthalmia* (Ranzani)

Only one adult of this species was injected, as it proved extremely fragile and was fixed in a dying condition only eight minutes after injection. The amount injected was 0.05 cc. of a 1:1000 solution of adrenalin. The immediate reaction was a dispersion of the pigment granules of the xanthophores. One minute later, the melanophores of the meninges seen through the top of the head showed a concentration of melanin granules. However, the black tip of the nose and the black anterior edge of the dorsal fin remained

dark, the melanophores still in a dispersed condition. No other gross change was visible.

The fish was examined immediately after formalin fixation under the dissecting microscope. Some of the dermal melanophores in the head region showed slight concentration of granules, but the rest appeared unaffected by the adrenalin. The magnification showed that the brain area, which had appeared snow white after the concentration of the melanophores, was covered with meninges in which all the iridophores were in their most brilliant condition. The area covered by this brilliance appeared larger than normal, so that it seems possible that there is some dispersion of guanin crystals within these cells.



TABLE 2  
EFFECTS OF HORMONE INJECTIONS ON THE PIGMENTATION OF VARIOUS TELEOSTS

| Species                         | Weight,<br>in Grams | Length,<br>in Mm. | Hormone and Dosage                     | Pigmentary Reaction   | Remarks                     |
|---------------------------------|---------------------|-------------------|--|---|-----------------------------|
| <i>Harengula macrophthalmus</i> |                     | (Adult)           | 0.05 cc. adrenalin 1:1000              | Melanophores concentrated; xanthophores and iridophores dispersed | Died 8 minutes after injury |
| <i>Ameiurus nebulosus</i>       | 85.0                | 163               | 0.20 cc. adrenalin 1:1000              | Melanophores concentrated   |                             |
|                                 | 23.0                | 99                | 0.10 cc. adrenalin 1:1000              | Melanophores concentrated   |                             |
|                                 | 229.2               | —                 | 55 mg. MSH                             | Melanophores dispersed  |                             |
| <i>Cyprinodon baconii</i>       |                     | (Small)           | 0.025 cc. adrenalin 1:1000             | Melanophores concentrated   |                             |
|                                 |                     | (Adult)           | 0.05 cc. adrenalin 1:1000              | Melanophores concentrated   | Died after 24 hours         |
| <i>Gambusia</i> sp.             | 1.9                 | 32                | 0.5 mg. MSH                            | No effects  |                             |
|                                 | 2.4                 | 33                | 0.5 mg. MSH                            | No effects  |                             |
|                                 | 0.5                 | 25                | 0.025 intermedin 4000 units per cc.    | Melanophores, xanthophores, and erythrophores dispersed           |                             |
|                                 | 0.5                 | 24                | 0.025 intermedin 4000 units per cc.    | Melanophores and xanthophores dispersed                           |                             |
| <i>Astyanax mexicanus</i>       |                     | (Adult)           | 1 mg. MSH                              | Melanophores and xanthophores dispersed                           | Died after 2 hours          |
|                                 | 1.8                 | 38                | 1.5 mg. MSH                            | Melanophores dispersed  |                             |
|                                 | 2.2                 | 43                | 1.5 mg. MSH                            | Melanophores dispersed  |                             |
|                                 | 2.1                 | 43                | 1.5 mg. MSH                            | Melanophores dispersed  |                             |
|                                 | 1.5                 | 41                | 1.5 mg. MSH                            | Melanophores dispersed  |                             |
| <i>Strongylura notata</i>       | —                   | 115               | 0.05 cc. adrenalin 1:1000              | Melanophores concentrated, xanthophores dispersed                 | Died after 1 hour           |
| <i>Atherina stipes</i>          | 42.7                | 273               | 0.5 cc. intermedin 4000 units per cc.  | No effects  |                             |
|                                 | —                   | (Adult)           | 0.05 cc. adrenalin 1:1000              | Melanophores concentrated   |                             |
|                                 | —                   | 64                | 0.05 cc. adrenalin 1:10,000            | No results  |                             |
|                                 | 2.4                 | 63                | 0.05 cc. intermedin 4000 units per cc. | Melanophores dispersed  |                             |
|                                 | 3.1                 | 56                | 1 mg. MSH                              | Melanophores dispersed  |                             |
| <i>Mugil trichodon</i>          |                     | (Juvenile)        | 0.025 cc. adrenalin 1:1000             | Melanophores and xanthophores concentrated                        |                             |
| <i>Sphyaena barracuda</i>       |                     | (Small)           | 0.20 cc. adrenalin 1:10,000            | No effects  |                             |
|                                 |                     | (Small)           | 0.20 cc. adrenalin 1:5000              | No effects  |                             |
|                                 |                     | (Small)           | 0.10 cc. adrenalin 1:5000              | No effects  |                             |
|                                 | —                   | 123               | 0.10 cc. adrenalin 1:1000              | Melanophores concentrated in iris only                            | Dead 24 hours later         |

TABLE 2—(Continued)

| Species                        | Weight,<br>in Grams | Length,<br>in Mm.  | Hormone and Dosage                     | Pigmentary Reaction   | Remarks             |
|--------------------------------|---------------------|--------------------|--|---|---------------------|
|                                |                     | (Small)            | 0.10 cc. adrenalin 1:1000              | Melanophores concentrated in iris only                            |                     |
| <i>Eucinostomus gula</i>       | 36.8                | 175                | 0.50 cc. intermedin 4000 units per cc. | No results  |                     |
|                                | 10.0                | 77                 | 0.10 cc. adrenalin 1:1000              | Melanophores concentrated in iris only                            | Dead 24 hours later |
| <i>Gerres cinereus</i>         | 154.0               | 183                | 0.35 cc. adrenalin 1:1000              | Melanophores concentrated in iris only                            |                     |
| <i>Apogonichthys stellatus</i> | 91.7                | 157                | 0.5 cc. intermedin 4000 units per cc.  | Xanthophores dispersed  |                     |
| <i>Pseudupeneus maculatus</i>  | —                   | —                  | 0.025 cc. adrenalin 1:1000             | Melanophores concentrated   |                     |
|                                | 106.5               | 178                | 0.50 cc. intermedin 2000 units per cc. | Erythrophores and xanthophores dispersed                          |                     |
| <i>Haemulon melanurum</i>      | 115.0               | 170                | 0.25 cc. adrenalin 1:1000              | Melanophores concentrated in iris only                            |                     |
|                                |                     | (Similar to above) | 0.20 cc. adrenalin 1:1000              | Melanophores concentrated in iris only                            |                     |
| <i>Haemulon sciurus</i>        | 127.8               | 157                | 1.3 cc. adrenalin 1:1000               | Melanophores concentrated   |                     |
| <i>Epinephalus striatus</i>    | 99.0                | 270                | 0.50 cc. intermedin 4000 units per cc. | Xanthophores dispersed  |                     |
|                                | 260                 |                    | 5.0 cc. adrenalin 1:1000               | Melanophores concentrated   | Dead 24 hours later |
|                                | 779                 | 2921               | 1.0 cc. adrenalin 1:1000               | No results  |                     |
|                                | 779                 | 2921               | 2.5 cc. adrenalin 1:1000               | Melanophores concentrated, xanthophores and iridophores dispersed |                     |
| <i>Lutianus apodus</i>         | 244                 | 1968               | 0.70 cc. adrenalin 1:1000              | Melanophores concentrated in iris only, xanthophores dispersed    | Dead 24 hours later |
| <i>Chaetodon striatus</i>      | 12.6                | 72                 | 3.0 mg. MSH                            | Xanthophores dispersed  |                     |
|                                | 62.0                | 113                | 0.7 cc. adrenalin 1:1000               | Melanophores concentrated in iris only                            |                     |
| <i>Chaetodon capistratus</i>   | 80.0                | 117                | 15.0 mg. MSH                           | Xanthophores dispersed  |                     |
|                                | 73.0                |                    | 0.7 cc. adrenalin 1:1000               | Melanophores concentrated in iris only                            |                     |
| <i>Angelichthys ciliaris</i>   | 70.0                | 117                | 0.5 cc. adrenalin 1:1000               | No results  |                     |
| <i>Pomacanthus paru</i>        | 32.0                | 86                 | 0.5 cc. adrenalin 1:1000               | No results  |                     |
| <i>Acanthurus caeruleus</i>    | 68.0                | 118                | 0.5 cc. adrenalin 1:1000               | No results  |                     |

TABLE 2—(Continued)

| Species                         | Weight,<br>in Grams    | Length,<br>in Mm. | Hormone and Dosage  | Pigmentary Reaction  | Remarks |
|---------------------------------|------------------------|-------------------|---|--|---------|
| <i>Thalassoma bifasciatum</i>   |                        | (Small male)      | 0.05 cc. adrenalin 1:1000   | Melanophores concentrated in iris only   |         |
|                                 |                        | (Small female)    | 0.03 cc. adrenalin 1:1000   | Melanin concentrated in iris only, xanthophores dispersed                                      |         |
| <i>Iridio biniatta</i>          | 116.3                  | 103<br>(Adult)    | 0.2 cc. intermedin 2000 units per cc.<br>0.15 cc. adrenalin 1:1000              | No results<br>Melanophores concentrated in iris only, erythrophores and xanthophores dispersed |         |
| <i>Sparisoma abigaardi</i>      | 61.0<br>12.3<br>4400.0 | 148<br>92<br>2603 | 0.25 cc. intermedin 2000 units per cc.<br>3 mg. MSH<br>1.6 cc. adrenalin 1:1000 | Erythrophores dispersed<br>Erythrophores dispersed<br>Melanophores concentrated in iris only   |         |
| <i>Pomacentrus leucostictus</i> | 20.0                   | 77                | 0.75 cc. adrenalin 1:1000   | Melanophores concentrated in iris and area of body cavity                                      |         |
|                                 | 12.0                   | 67                | 0.10 cc. adrenalin 1:1000   | Melanophores concentrated in iris only   |         |
| <i>Abudefduf saxatilis</i>      | 70.0                   | 1143              | 0.15 cc. adrenalin 1:1000   | Melanophores concentrated in iris and dermis, not in epidermis, xanthophores dispersed         |         |
| <i>Carapus bermudensis</i>      | —                      | —                 | 0.025 cc. adrenalin 1:1000  | Melanophores concentrated, erythrophores and iridophores dispersed                             |         |
| <i>Eretelis smaragdus</i>       | 5.0                    | 99                | 0.05 cc. adrenalin 1:1000   | Melanophores concentrated  |         |
| <i>Bathygobius soborator</i>    | —                      | 68                | 0.05 cc. adrenalin 1:1000   | Melanophores concentrated, xanthophores first dispersed, then concentrated                     |         |
|                                 | —                      | —                 | 0.20 cc. intermedin 2000 units per cc.<br>4 mg. MSH                             | Xanthophores dispersed<br>Xanthophores dispersed   |         |
| <i>Opsanus</i> sp.              | 16.6                   | 88                | 0.05 cc. adrenalin 1:1000   | Melanophores concentrated  |         |
| <i>Balistes capricus</i>        | —<br>148.0             | —<br>1460         | 0.30 cc. adrenalin 1:1000   | Melanophores concentrated in iris and about mouth  |         |
| <i>Monacanthus ciliatus</i>     | 5.2                    | 56                | 0.05 cc. intermedin 4000 units per cc.  | No results   |         |
|                                 | 6.0                    | —                 | 2 mg. MSH   | No results   |         |
| <i>Ogcocephalus radiatus</i>    | 74.0                   | 135               | 0.1 cc. adrenalin 1:1000  | Melanophores concentrated in iris  |         |
|                                 | 74.0                   | 135               | 0.2 cc. adrenalin 1:1000  | Melanophores concentrated in iris, some white patches on body                                  |         |

## ORDER NEMATOGNATHI

## FAMILY SILURIDAE

*Ameiurus nebulosus* (Rafinesque)

Two catfish were injected. The larger one, which received 0.2 cc. of adrenalin 1:1000 measured 163 mm. in standard length, weighed 85 grams, and was a light gray in color. A second, smaller, fish, which received 0.1 cc. of adrenalin 1:1000, measured 99 mm. in standard length and weighed 23 grams. This fish was almost black in color. The differences in coloration between the two fishes resulted from different backgrounds and lighting conditions in the two separate tanks in which they had been living.

Four minutes after injection the larger fish showed some lightening, particularly about the mouth, and after six minutes a mottled gray and light coloration appeared over the body. Ten minutes after injection the fish was only slightly lighter than at the beginning of the experiment. Twenty minutes after injection the entire fish was a pinkish white except for the tips of the barbels which remained dark throughout the experiment. Four minutes after injection the small, blacker fish appeared gray, and no further lightening was seen after seven minutes. One hour after injection, this fish also had become a pinkish white, with black-tipped barbels. In both fishes the reaction appeared to be a slow one.

The reaction of the iris was difficult to determine, as it is normally light in this species. However, after the fish had become pink, the sclera could be seen with a magnifying glass through the translucent skin around the eye and was observed to be extremely light.

Four hours after injection the color had returned over the dorsal surface of the larger fish, although the smaller fish was still whitish. By the end of five hours both fishes had regained their original coloration.

One catfish weighing 229.2 grams was injected with 55 milligrams of Armour's melanophore-stimulating hormone in 1 cc. of 0.6 per cent saline solution. Within a few minutes the fish showed definite darkening over the dorsal region. This darkening, caused by dispersion of pigment granules in the melanophores, increased gradually, until the whole fish, except for the ventral area where

there are no melanophores, was completely black. Normal coloration did not return until the following day.

## ORDER CYPRINODONTES

## FAMILY CYPRINODONTIDAE

*Cyprinodon bacon* (Breder)

One rather small specimen was injected with 0.025 cc. of a 1:1000 solution of adrenalin and placed in an aquarium with a non-injected control.

After two minutes the iris appeared white and the coloration of the entire body was slightly paler than that of the control. After six minutes the injected fish was still slightly paler than the control. After 14 minutes the anterior portion of the head still remained pale, while the rest of the body coloration was the same as that shown by the control. This white mask effect was still unchanged after 34 minutes, but after 64 minutes it had been reduced to a small area between the eyes and the tip of the snout. Shortly thereafter the injected animal was indistinguishable from the non-injected fish.

Obviously the dose given was not a very strong one, as its effect began to wear off very quickly. However, the reaction was prompt and conformed to the accepted idea of the teleost reaction to adrenalin by a concentration of pigment granules within the dermal melanophores. That the epineural melanophores reacted more strongly than the dermal ones is shown by the more intense reaction of the iris.

Another individual, a full grown male, was injected with 0.05 cc. of adrenalin 1:1000. Within 45 seconds the iris and the scales about the site of the injection became white. After one minute and 45 seconds a peculiar mottling pattern was seen, with darkish patches on the dorsal and lateral surfaces. The lateral dark patches gradually gave way to white, leaving the dorsal surface dark enough to show the blue color. Three minutes after injection the dorsal surface was still fairly dark, and the dark patterns of the dorsal, anal, and caudal fins remained intact.

Thirteen minutes after injection the fish was all white except for the base of the dorsal fin and the black tip of the caudal. The area about the mouth became very pink owing to

the increased visibility of the underlying blood vessels.

After 30 minutes the pattern on the fins still remained intact. The fish by this time showed abnormal behavior, apparently being unable to keep its equilibrium. The next morning the fish was found dead.

One adult male and one adult female were each injected with approximately 0.5 milligram of melanophore-dispersing hormone. The male measured 32 mm. in standard length and weighed 1.9 grams. The female measured 33 mm. in standard length and weighed 2.4 grams. The injected fishes were slightly darker when returned to the stock tank than the other fishes, owing to the light urethane anesthesia used at the time of weighing and injecting. Ten minutes after injection both fishes were indistinguishable from their fellows and remained normal in coloration thereafter. No effects of MSH were noted.

#### FAMILY POECILIIDAE

##### *Gambusia* sp.

One male and one female *Gambusia* were each injected with 0.025 cc. intermedin (Choay, 4000 units per cubic centimeter). The standard length of the male was 25 mm. and of the female 24 mm. Each weighed approximately 0.5 gram. They were observed under a dissecting microscope on a white background. Before injection they were in a very light color phase; melanophores were punctate. Immediately after injection the granules of the melanophores became more dispersed although not to their full extent. The orange color on the dorsal fin of the male, which was not seen before injection, became prominent owing to the dispersion of pigment granules in the xanthophores. Both of the injected fishes were darker than the non-injected control which was in the same container. Observation of the area on the top of the head showed that pigment cells when in the dispersed condition do not completely cover the pineal area. There is still a differential between protected and unprotected areas. The pigmentary reaction could be overcome by fright, for when the fish were disturbed in the dish with a net, the injected animals became as pale as the controls.

One adult female was injected with 0.03 cc. of saline solution in which was dissolved

1 milligram of melanophore-stimulating hormone (Armour). The injected fish and a non-injected control were observed under the dissecting microscope against a white background. After five minutes the injected fish showed xanthophores with dispersed pigment in the meninges, and some dermal melanophores also showed dispersion of pigment granules. Ten minutes after injection two dark patches of dispersed melanophores appeared on the dorsal surface of the fish, one anterior and one posterior to the dorsal fin. The pigment of melanophores in the fins was also in a more dispersed condition than in the control. After 15 minutes the injected fish had a much yellower cast all over than the control, indicating the dispersion of pigment in the dermal xanthophores. This reaction lasted for two hours; the injected fish then died.

#### ORDER SYNENTOGNATHI

##### FAMILY BELONIDAE

##### *Strongylura notata* (Poey)

This species is one that is too fragile to withstand the handling necessary for many experimental procedures. The specimen injected was a small fish, 115 mm. in standard length. It was first given a dose of 0.025 cc. of epinephrine 1:1000. After six minutes there was no visible change in coloration, and the iris still retained its normal color. After 11 minutes a lighter phase was noted momentarily, then the coloration became like that of the control.

A second injection of 0.05 cc. was given after 16 minutes, as there seemed to be a possibility that the first dose had been insufficient or had leaked out. One minute after the second injection the melanin granules of melanophores of the meninges became concentrated, and the meninges became white. The yellow of dispersed xanthophores was also apparent in the same region. After two minutes the iris and the back of the eyeballs, which can be plainly seen in this fish, became white. The body coloration was considerably lighter than the control, and the green coloration disappeared. The beak alone remained dark. The loss of the green color was the result of the concentration of melanin granules within the chromatophores of the dermis and of the peritoneum. After six minutes a few melanophores could again be seen in the meninges,

but the fish appeared to be dying. After 10 minutes both fishes were put in the dark room. Two hours later the injected fish was still lighter than the control, and both fishes were found resting on the bottom of the tank. Forty minutes after the last observation, both fishes were found dead.

One individual was injected with 0.5 cc. of intermedin (Choay, 4000 units per cubic centimeter). The standard length was 273 mm. and the weight 42.7 grams. The fish was placed in an outdoor tank with a white sand bottom in the sunlight. No change in coloration whatever was observed in this fish up to six hours after injection.

#### ORDER PERCESOCES

##### FAMILY ATHERINIDAE

##### *Atherina* (*Hepsetia*) *stipes* (Müller and Troschel)

This species is also very fragile, although somewhat less so than *Strongylura*. One specimen was injected with 0.05 cc. of adrenalin 1:1000. One minute after injection, the meninges, which can be seen through the transparent overlying tissues, became white. Three minutes after injection the tops of the eyeballs were white and the tips of the caudal fin still remained dusky, although they were somewhat lighter than those of the non-injected control. The normal greenish color disappeared. Five minutes after injection granules within the dermal melanophores of the body region did not appear much more concentrated than those of the control.

After 11 minutes the injected animal was fixed in formalin, and the control fish was also fixed after first being put in a urethane solution to disperse the melanophore granules. Examination under the dissecting microscope showed that the dermal melanophores of the adrenalin-injected fish were no more concentrated than those of the control subjected to the melanin-dispersing action of the urethane anesthesia. However, the black tail tips were lighter than those of the control, and the whole body appeared paler owing to the loss of the green coloration. As in *Strongylura*, the greenish color is probably caused by the presence of internal melanophores about nerves, blood vessels, and the peritoneum. The color would thus be lost when these pigment cells became concentrated.

A second specimen somewhat larger than the first (64 mm. in standard length) was in-

jected with 0.05 cc. of adrenalin 1:10,000. This dosage was presumably insufficient to cause any reaction, as the meninges were not affected, and over a black background the tail tips retained their black coloration.

One individual was injected with 0.05 cc. of intermedin (Choay, 4000 units per cubic centimeter). The fish appeared darker after injection, and the black edges to the scales were prominent. The reaction occurred very soon after injection. The standard length of the fish was 63 mm. and the weight 2.4 grams.

One *Atherina* measuring 56 mm. in standard length and weighing 3.1 grams was injected with approximately 1 milligram of melanophore-dispersing hormone. Within 10 minutes the pigment granules in melanophores around the mouth and chin and in the rows down the sides were partially dispersed. The caudal fin did not become black. The injected fish remained in the same coloration as the rest of its kind in the tank except as above noted. Three hours after injection the fish showed concentrated melanophores as did its companions.

##### FAMILY MUGILIDAE

##### *Mugil trichodon* (Poey)

One small specimen was injected with 0.025 cc. of adrenalin 1:1000. One minute after injection the granules in the meningeal melanophores became concentrated, and the area over the top of the brain appeared yellow as a result of the dispersion of pigment in the xanthophores. Three minutes after injection the whole animal appeared lighter than the non-injected control. All the melanophores, including the dermal ones, reacted to the adrenalin by concentration of the melanin granules. The area over the pineal appeared pink at this same time, owing to a secondary concentration of the xanthophores and to the absence of iridophores in the meninges of this species in the juvenile.

##### FAMILY SPHYRAENIDAE

##### *Sphyraena barracuda* (Walbaum)

Ten individuals of this species were used for experimental purposes. After having been injected with 0.2 cc. of adrenalin 1:10,000, one specimen showed an abnormal color phase; the dark bands of the pattern were not



obliterated but showed small black spots that gave a freckled appearance in certain areas. Over a white background the bands of the pattern remained visible and the snout was black. Over a dark background, the dark-banded pattern was more pronounced but not so bold as that shown by the control. The snout remained black, and the deep black lateral spots remained dark regardless of the background. One hour after injection this fish showed normal reactions to dark and light backgrounds.

It seems probable that the abnormal freckled appearance was a result of injury to scales or peripheral nerves incident to handling. Another fish injected with 0.2 cc. of spring water which had been used to dilute the adrenalin also showed the same abnormal freckling and black snout.

Two additional specimens were injected with adrenalin 1:5000, the larger receiving 0.2 cc. and the smaller 0.1 cc. The freckled appearance and black snout showed in three minutes but were not so pronounced as in the previous experiments. The two fishes were observed in an outdoor pool in which the light-colored sand bottom was covered in one area by a large piece of black paper. The fish could therefore be made to swim over a dark or a light background. When either of these injected fishes was driven over the black background it was able to darken the coloration within the same five seconds characteristic of the reaction of normal non-injected fish of this species. On the black background the fish that had received the larger dose showed such a dark coloration that the pattern was entirely eliminated, while the fish that received the smaller dose appeared considerably lighter over the same background, so that while the coloration was dark the banded pattern was still distinguishable. When driven back on the light background, both fishes assumed their light coloration within the same time shown by the non-injected controls. One hour after injection the fish that had received the lighter dose of adrenalin had assumed a normal coloration without the freckling and the black snout. After an additional half an hour the fish that had received the stronger dose again showed the normal coloration.

Another small barracuda, 123 mm. in

standard length, was injected with 0.1 cc. of adrenalin 1:1000. This fish was kept in a tank with a background so mottled that the fish normally maintained a coloration showing a plainly visible pattern. Immediately after injection both the dark and light bands of the pattern lightened but by no means disappeared. The iris became white and irregular in shape, although some semblance of the lateral stripe through the iris was maintained. Five minutes after the injection the dorsal surface of the fish showed a pinkish cast, possibly owing to some vascular congestion. Eleven minutes after injection the top of the head became black, not over the snout as was recorded for the fish that received diluted adrenalin, but between the nose and the posterior part of the eyes. The first band of the pattern behind the eye appeared somewhat faded. Twenty-two minutes after injection the iris was almost completely white, with only a vestige of the lateral stripe remaining. Two and a half hours after injection the normal coloration of the iris had returned, and the black head was lighter in color. The fish appeared completely normal four hours after injection but was found dead on the following morning.

Although this fish had received a massive and probably lethal dose of adrenalin, it was able to maintain the pattern throughout the duration of the experiment. The fish never showed so light a coloration as normal non-injected specimens can assume on a light background.

Still another experiment was performed on two additional fish, one of which received 0.1 cc. of adrenalin 1:1000. The second fish was used as a non-injected control. These two fishes were observed in an outdoor pool. Three minutes after injection both fishes were over the light background and showed a light color phase without pattern. Six minutes after injection both fishes were driven over the black background. The injected fish assumed the dark-banded pattern in approximately 10 seconds, about double the time shown by the control. The injected fish rested on the bottom; the control remained quiescent about 2 inches above the bottom. After 11 minutes the injected fish was driven over the light background, and the pattern was promptly eradicated. As the fish settled to the bottom,

the anterior half of the body came to rest over the black background. In this position the dark-banded pattern was assumed within five seconds. Eighteen minutes after injection, the iris of the injected fish was completely white, and the respiration of the fish as judged by the opercular movements was considerably slower than that of the control. Several hours later the injected and control fishes were indistinguishable, and both were alive the following morning. Plate 9, figure 1, is a photograph of these two fishes. The larger fish was injected with adrenalin. The white iris is the result of the adrenalin injection. The lighter body coloration is the result of some extrinsic factor, probably the proximity of the other fish.

A cut made in the lower half of the caudal fin of another small barracuda produced the caudal bands described by Fries (1943) for *Fundulus*. The darker band appeared within a few seconds after the cut was made and was still pronounced three days later. There appears to be no difference in the function of the nervous control of the melanophores in this species from one that reacts in the classic manner to adrenalin injection.

One individual was injected with 0.5 cc. of intermedin (Choay, 4000 units per cubic centimeter). The standard length was 175 mm. and the weight 36.8 grams. The fish was in a fairly dark phase when injected. It was placed in an outdoor tank with a white sand bottom in sunlight. The sides of the tank were fairly dark with an algal growth. Five minutes after injection the fish was in the same fairly dark phase with darker bands showing. Half an hour later the fish was still in a dark phase but without bands. At this time a fish of another species was added to the tank, which apparently disturbed the barracuda and he assumed his lightest phase without markings of any kind. The change was as rapid as that shown by any non-injected fish of the same species. After two hours he had returned to a fairly dark phase without bands. A hand passed over the surface of the water caused the dark bands to appear. At the end of six hours the fish was still in the same dark phase without bands. It seems obvious that if the intermedin had any effect, it was only when the fish was in an undisturbed, resting condition, and the

effects could be rapidly overcome if the situation demanded that the pigmentation be changed.

## ORDER PERCOIDEA

### FAMILY GERRIDAE

#### *Eucinostomus gula* (Cuvier and Valenciennes)

One specimen was injected with 0.1 cc. of adrenalin 1:1000. The fish weighed 10 grams and measured 77 mm. in standard length. This species is too fragile for much experimental work; it is almost impossible to handle without removing so many scales that the physiology of the fish is changed before any experimental procedures have been undertaken.

Immediately after injection the fish assumed a very pale phase, but the non-injected control showed a color phase that was even lighter. The caudal fin of the injected fish remained darker than that of the control. Two minutes after injection the iris of the injected fish was silvery white. A very white area was seen over the snout and head between the eyes. The swimming movements of the fish were abnormal at this time, producing a somersaulting pattern. Five minutes after injection, the top of the snout anterior to the white area became black. The fish had by this time resumed normal swimming movements. A somewhat darker coloration appeared over the dorsal surface. The control was also faintly dark at this time and showed faint vertical bars. Thirty minutes after injection the fish was very light except for the black tip of the snout and a dark mottling over the midline. The black tail tips that are sometimes seen on this species when they are over a white coral sand background were not visible on either injected or control fish at any time during the experiment. The following morning the injected fish was found dead.

#### *Gerres cinereus* (Walbaum)

One specimen was injected with 0.35 cc. of adrenalin 1:1000. The fish weighed 154 grams and measured 183 mm. in standard length.

Immediately after injection the fish showed vertical gray bars, while the non-injected control showed longitudinal stripes. Seven minutes after injection the iris of the injected fish became silvery white. Both this fish and

the control showed vertical gray bars at this time. Twelve minutes after injection the experimental fish appeared much lighter than the control, but this reaction was reversed after another three minutes when the control became the lighter fish. In the injected fish two spots on the dorsal aspect of the iris remained fairly dark throughout the experiment, and the yellow color of the ventral, anal, and dorsal fins remained unaffected. Twenty minutes after injection the caudal fins of both fishes were dusky, and the same body coloration was shown by both fishes.

The behavior of the fishes was unusual. After the initial thrashing about on being put into the 15-gallon observation tank, they settled to the bottom and moved hardly at all. They could be pushed around into any convenient position with a glass rod, and in fact this was done for purposes of photography.

Thirty minutes after injection there was still no discernible difference between the body coloration of one fish and that of the other; the injected fish was distinguishable from the control only by the silvery white iris.

One individual was injected with 0.5 cc. of intermedin (Choay, 4000 units per cubic centimeter). The standard length was 157 mm., and the weight was 91.7 grams. The injected fish was placed in an outside pool with a white sand bottom in sunlight. Five minutes after injection the fish was in a fairly dark phase, with yellow color prominent in the pelvic fins. However, there was not much change from the condition before injection, and the coloration remained unchanged for six hours.

#### FAMILY APOGONIDAE

##### *Apogonichthys stellatus* Cope

One specimen was injected with 0.025 cc. of adrenalin 1:1000. Three minutes later the iris was white, as was the outside covering of the eyeballs. Because these could be seen through the transparent tissues, they made a broad, silvery white band across the top of the head. During the next two minutes the lightening of the whole fish proceeded posteriorly, until the fish showed a very pale phase over the entire body. Seven minutes after injection the sharp black and white pattern was still seen on the ventral fins. In the

region of the body anterior to the ventral fins, the skin contained more iridophores than posteriorly. Under the influence of adrenalin a much lighter appearance is imparted to the anterior region in contrast to the more transparent character of the posterior part of the body. Nine minutes after injection the tips of the dorsal and caudal fins and the black spots of the ventrals were still black.

After 43 minutes the great majority of the body melanophores were concentrated to points. Scattered single cells were occasionally seen in the dispersed state, either unaffected by the adrenalin or dispersed after the hormone had ceased to be effective. At this time also a sharp line of demarcation was noted between light and dark coloration in the ventral, dorsal, anal, and caudal fins. Either the adrenalin had not taken effect in these fins, or the effect was wearing off and the color was returning. After 53 minutes the fish and its control were photographed (pl. 10, fig. 1). The picture shows that the color was returning over the dorsal surface at that time.

#### FAMILY MULLIDAE

##### *Pseudupeneus maculatus* (Bloch)

One individual was injected with 0.5 cc. of intermedin (Choay, 2000 units per cubic centimeter). The standard length was 178 mm., and the weight 106.5 grams. About a minute after injection the fish assumed a red coloration. The fish was observed in the aquarium in which it had been accustomed to living and in which it frequently assumed this red coloration. However, five minutes after injection dark bands appeared over the dorsal surface. These could be made to disappear when the fish was disturbed, as by passing a hand across the front of the tank. Twenty minutes after injection the fish was in a brilliantly colored red phase, with three lateral black patches. Yellow color made by dispersed xanthophores was prominent in the caudal fin. When frightened, the fish promptly lost all color. The dark red color was maintained up to an hour after injection when it gradually began to disappear. Four hours after injection the fish had reassumed its normal pale phase with three lateral black spots, although a yellow color was still prominent along the sides. Six hours after injection

the fish was in its palest phase, with all signs of chromatophore dispersion obliterated.

FAMILY **SERRANIDAE**

***Epinephalus striatus*** (Bloch)

One specimen weighing 260 grams and measuring 270 mm. was injected with 5 cc. of adrenalin 1:1000. This proved to be a lethal dose. One and one-half minutes after injection small white patches appeared over the lateral, dorsal, and ventral surfaces. After two minutes the iris began to whiten. After seven minutes the iris was completely white. Except for white patches around the site of the injection, the fish showed a dark color phase against the dark bottom of the tank. After 15 minutes the iris and eyeball were so white that the eye appeared pink when observed through the pupil. After 30 minutes the animal appeared almost pure opaque white, with a few black spots over the operculum. The edges of the fins were also still dark, and the black quadrangle on the caudal peduncle showed white spots in it. Five hours later the fish appeared the same, and after seven hours the quadrangle on the caudal peduncle was all white except for a small black spot. The next morning this fish was dead, still in the opaque white phase.

Another specimen weighing 779 grams and measuring 290 mm. in standard length was injected with 1.0 cc. of adrenalin 1:1000. This dosage proved to be ineffective. The following day the same individual was given 2.5 cc. of adrenalin. The fish was in a large outdoor tank with a dark bottom and was in an all-over dark brown color phase when injected. After the injection it was put in an observation tank with a light bottom. In 10 seconds the banded pattern was produced by the fish and after one minute and 45 seconds the fish had lightened considerably but without erasing the banded pattern. This first color reaction was probably due to handling or to the change in coloration of the bottom. Two minutes after injection the effects of the adrenalin began to appear. The dorsal aspect of the iris lightened and small white patches appeared over the entire body in both the light and dark bands. The fish was now in a very light phase, although the banded pattern still showed faintly. The black quadrangle on the caudal peduncle which is characteristic of this species remained dark as did two black

patches on the dorsal and ventral edges of the caudal fin and the ventral edge of the anal fin. The iris now appeared a yellow-orange in color.

Five and one-half minutes after injection the banded pattern again appeared more pronounced, with the quadrangle on the caudal peduncle very black. After seven minutes the dark brown all-over color phase appeared.

Ten minutes after injection, half of the bottom of the tank was covered with black paper so that the reactions of the fish over a dark or light bottom could be studied by easing the fish over either bottom. At this time the fish had not lost its ability to respond to the background; it became very dark when over the black bottom and lightened when over the light sand bottom. The fish therefore could change the color phase at will. The black quadrangle remained intact regardless of the color phase.

Fourteen minutes after injection the iris still appeared red-orange, the xanthophores unaffected by the adrenalin. Opaque white patches appeared in two small areas of the iris—one small area at the edge of the black quadrangle, and a fairly large area at the site of injection.

Seventeen minutes after injection the fish was still able to change its color phase, seeming to prefer the dark background where it maintained the dark brown phase. However, when it settled on the white sand the banded pattern was observed. The iris was at this stage opaque white around the entire periphery and in some places at the periphery of the pupil.

After 22 minutes the banded pattern was observed when the fish was on the dark background.

Forty minutes after injection the iris was nearly all opaque white. An area over the head between the eyes and extending a little way posteriorly became a pale yellow. Opaque white patches over the entire body gave a freckled appearance to the fish. The whiteness over the head region was not bilaterally symmetrical; on the left side the whiteness extended down over nearly the entire operculum.

After 47 minutes the white areas showed no yellow tinges, indicating that pigment in the xanthophores was concentrated by the ad-

renalin. The fish which was lying on the black background showed white patches over the entire body; all control over color phases had been lost at this time. The black quadrangle on the caudal peduncle was still unaffected, except that the edges of the pattern were hazy instead of sharp.

After three and one-half hours the fish was still opaque white. The iris was completely white, except for small yellowish patches on the dorsal aspect. The entire fish was now white, save for small black patches on the dorsal surface and the black quadrangle. At both six and seven hours after injection the fish remained unchanged.

At seven hours the chromatophores were observed under the low power of the dissecting microscope. Melanophores were concentrated to points. The milk-white appearance of the fish was induced by the presence of leucophores uncovered by the concentration of the melanin in the overlying melanophores. On the dorsal fin, where there remained some dark brown color, the melanophores were not so concentrated but nevertheless showed no branching. The yellow edge of the dorsal fin was made by dispersed pigment in xanthophores.

Eleven and one-half hours after injection the banded pattern was reestablished and color had returned to the iris. However, the light bands were somewhat lighter than normal. The next morning the fish had returned to normal coloration.

#### FAMILY LUTIANIDAE

##### *Lutianus apodus* (Walbaum)

One specimen weighing 244 grams and measuring  $7\frac{3}{4}$  inches in standard length was injected with 0.7 cc. of adrenalin 1:1000. This fish had been living for approximately five weeks in an outdoor pool. Removal to a smaller indoor tank for observation purposes caused the fish to become dark all over, eliminating the barred pattern but retaining the yellow fins.

There was no immediate reaction to the injection. After one-half hour the iris showed irregular white patches at the periphery of the pupil. A light color was also shown over the top of the head between the eyes and a short distance anterior and posterior to the eye region. Forty minutes after injection a

peculiar freckled appearance was seen over the entire body. The iris was not completely white, as it contained many xanthophores unaffected by the adrenalin injection. The iris melanophores were concentrated. Fifty minutes after injection the fish appeared to be in a light phase, except for a blackish mottling caused by some of the scale melanophores that were still dispersed. The fins remained yellow, with xanthophores unaffected by the adrenalin. After four hours the coloration of the body was normal. The iris, however, was still white, except for the dorsal aspect where normal coloration had returned.

This fish was found dead the next morning. Death was possibly not due to the adrenalin injection but to confinement in too small a tank overnight.

One small schoolmaster measuring 72 mm. in standard length and weighing 12.6 grams was injected with 3 milligrams of melanophore-dispersing hormone. Ten minutes after injection the fish was much yellower than its companions and also a slightly darker gray. The black bar through the eye disappeared. The darker gray coloration was not by any means the darkest phase this fish is capable of assuming. One half-hour after injection the coloration had returned to normal. However, 18 hours later the fish was much yellower than its fellows living in the same tank over the same background, and this slightly yellower coloration distinguished the injected fish for about four days.

#### FAMILY HAEMULIDAE

##### *Haemulon melanurum* (Linnaeus)

Several fishes of this species were used for experimentation, as the deep black pattern over the dorsal surface and fin made convenient material for study. The first specimen injected, which received 0.25 cc. of adrenalin 1:1000, weighed 115 grams and measured 170 mm. in standard length. Five minutes after the injection the fish had become dark in coloration, the black back was only a darker gray than the rest of the body, and the head was dark, particularly around and between the eyes. Fifteen minutes after injection the fish was still a dark gray, with a peculiar, all-over, white, freckled appearance which was possibly a result of injury to scales in handling. Twenty minutes after

injection the iris showed a silvery white and ragged appearance. The ordinarily dark pattern of the iris that makes a continuation of the lateral stripe through the eye was thus obliterated. The fish was otherwise a dark gray and sometimes could be seen to darken the back almost to black. After 30 minutes a non-injected control was put in the same tank, and the control assumed the same coloration as the injected fish except for the iris (pl. 9, fig. 2).

Thirty-five minutes after injection the glass front of the tank was cleaned preparatory to the taking of photographs. During this process the fishes remained quiet and showed no undue excitement, but both put on an all-over, dark gray coloration, darkening the light areas and lightening the black back until the pattern was almost obliterated. The eyes of the injected fish were much more conspicuous than those of the control and were not responsive to the changes in coloration that the rest of the body underwent.

After one hour the lateral stripes began to reappear in the iris of the injected fish, and after another half hour the eyes of both control and injected fishes were alike in coloration. Two and one-half hours after the start of the experiment both fishes showed their normal or most usual color phase, that is, light with yellow stripes and a dense black back.

Another fish of the same species, similar in size, was given 0.2 cc. of adrenalin 1:1000 and was immediately placed in a tank in a dark room with a non-injected control. Observations were made at 20 and 40 minutes after injection, and in each case both fishes presented the dark-striped nighttime phase. At both observation times the iris of the injected fish was white, and the control animal

appeared to be able to lighten his coloration more quickly than the injected one when the light was turned on.

Four additional fishes were injected with different dosages of adrenalin 1:1000. The fishes were weighed, and the dosages were calculated so that the four fishes received, respectively, injections of 0.125 cc., 0.25 cc., 0.5 cc. and 1.0 cc. per 100 grams of body weight. (See table 3.)

The four fishes, together with a non-injected control, were observed in a large outdoor pool, and the separate injected fishes were identified by means of different colored threads tied loosely about the caudal peduncle.

Three minutes after injection the iris of no. 4, the fish that received the largest dose, had whitened. At 10 minutes after injection, the iris of no. 3 showed a beginning of whitening, mostly on the ventral segment of the iris. At this time the two fishes with the smallest dosages showed the darkest coloration, with an intensification of the color of the black back. After another minute, no. 3 showed the black back; thus at this time they were all black-backed except the fish with the largest dose.

Twelve minutes after injection the iris of no. 2 began to whiten, and again the process was noted to start from the most ventral portion of the iris. The iris of the fish receiving the smallest dosage of adrenalin never whitened but remained indistinguishable from that of the control throughout the experiment.

Fifteen minutes after injection a graded position regarding dosage and coloration of the fishes was shown, with the control intermediate in coloring. The fish with the smallest dose was the darkest; the fish with

TABLE 3  
WEIGHTS OF FOUR SPECIMENS OF *Haemulon melanurum* (LINNAEUS) AND THE  
CORRESPONDING INJECTIONS OF ADRENALIN

| Fish No. | Weight (in Grams) | Factor  | Cubic Centimeters<br>per 100 Grams | Cubic Centimeters<br>Actually Injected |
|----------|-------------------|---------|------------------------------------|--|
| 1        | 102.5             | 0.00125 | 0.125                              | 0.13                                   |
| 2        | 72.8              | 0.0025  | 0.25                               | 0.18                                   |
| 3        | 135.5             | 0.005   | 0.50                               | 0.68                                   |
| 4        | 127.8             | 0.01    | 1.00                               | 1.30                                   |



the largest dose, the lightest. The extent of the iris whitening was graded in the same way. After 20 minutes, the color of the iris had returned somewhat to nos. 2 and 3. The normal color was seen immediately around the pupil and extended about halfway to the outer rim of the iris. After another half hour the iris of no. 2 was completely normal, but the irises of the other three fishes remained unchanged. Eighty minutes after injection all the fishes showed a completely normal coloration except no. 4 which exhibited the black freckled appearance noted in the adrenaline-injected barracuda.

***Haemulon sciurus* (Shaw)**

One individual was injected with 0.5 cc. of intermedin (Choay, 4000 units per cubic centimeter). The standard length was 157 mm. and the weight 99 grams. After injection the fish was placed in an outside pool with a white sand bottom in sunlight. Five minutes later it appeared, if anything, lighter than before the injection. A control, non-injected fish of the same species was added to the tank after half an hour, and no difference in coloration was observed between the two. However, six hours after the injection the injected fish was yellower than the control, an indication of dispersion of pigment granules in the xanthophores.

**ORDER SQUAMIPINNES**

**FAMILY CHAETODONTIDAE**

***Chaetodon striatus* Linnaeus**

One specimen that weighed 62 grams and measured 113 mm. in standard length was injected with 0.7 cc. of adrenalin 1:1000. Twenty seconds after injection the iris showed white patches in the black parts which form a continuation of the black band of the pattern that is continued from the dorsal to the ventral aspect of the fish. This was the only reaction shown by this fish to administration of adrenalin. The bold pattern made by the distribution of pigment cells in the skin was completely unaffected by the injection.

One individual weighing 80 grams was injected with 15 milligrams of melanophore-stimulating hormone dissolved in 0.4 cc. saline solution. At the time of injection the fish was in a strong black and white banded phase.

The normal coloration of this fish includes a strong yellow patch banded with blue which extends just above the snout, between and above the eyes. After 10 minutes the coloration of the fish was unchanged, but after one-half hour the yellow patch on the forehead and the yellow edges of the caudal and anal fins showed a slightly stronger coloration than those of the non-injected control. An hour later a pale yellow color was seen to be spread over the ventral surface of the body of the fish, which lasted for about five hours before it began to fade. At no time after injection was any reaction of melanophores noted, although this species is able to darken the white bands considerably under appropriate conditions of dark backgrounds.

***Chaetodon capistratus* Linnaeus**

One specimen weighing 73 grams and measuring 117 mm. in standard length was injected with 0.7 cc. of adrenalin 1:1000. Twenty seconds after injection the iris became completely white; the black stripe running through the iris was obliterated. No other reaction to the adrenalin was observed, and the rest of the dermal pattern was unaltered.

***Angelichthys ciliaris* (Linnaeus)**

One specimen weighing 70 grams and measuring 117 mm. in standard length was injected with 0.5 cc. of adrenalin 1:1000. No effect of the adrenalin was noted, even in the iris.

***Pomacanthus paru* (Bloch)**

One specimen weighing 32 grams and measuring 86 mm. in standard length was injected with 0.5 cc. of adrenalin 1:1000. No effect of the injection was noted, even in the iris.

**FAMILY ACANTHURIDAE**

***Acanthurus caeruleus* (Bloch and Schneider)**

One specimen weighing 68 grams and measuring 118 mm. in standard length was injected with 0.5 cc. of adrenalin 1:1000. This fish paled within five seconds of injection, and within two minutes the fins also appeared pale except on the extreme periphery. After seven minutes the fish darkened to its usual deep blue color. After 16 minutes the fish again showed the pale phase, and at the

same time the iris appeared somewhat lighter. These reactions did not depend on the adrenalin injection, because the fish paled only when disturbed, as by a person walking by the observation tank, and darkened at will, although under the influence of the adrenalin injection.

#### ORDER PHARYNGOGNATHI

##### FAMILY LABRIDAE

###### *Thalassoma bifasciatum* (Bloch)

One male was injected with 0.05 cc., and one female, smaller than the male, was injected with 0.03 cc., of adrenalin 1:1000. The pigmentation of the female responded to the adrenalin more than did that of the male. However, the female may have received a greater dose per gram of body weight. One minute after injection the pigment granules in the xanthophores of the female were dispersed, so that the dorsal surface was yellow, and at the same time the fish showed a continuous dark lateral stripe as compared with the female control that showed a broken lateral stripe and no yellow color on the dorsum. The male had assumed a mottled appearance, but the color pattern remained distinct. Two minutes after injection the head of the female became white, as a result not only of the concentration of pigment granules in the melanophores of the meninges but also of the dermal melanophores, so that the pattern was obliterated from the head region. The iris was white in both injected fishes. The male showed a clearer space over the head in the blue area, and light mottlings appeared over the entire body. Four minutes after injection only remnants of the lateral stripe were left on the female. The male at this time was seen to be lighter than the control, but the blue color was never obliterated completely. Seven minutes after injection no further changes had appeared.

One male was injected with 0.20 cc. of intermedin (Choay, 2000 units per cubic centimeter). The standard length was 103 mm., and the weight was 116.3 grams. Throughout a five-hour observation period there was no change in coloration whatever.

###### *Iridio bivattata* (Bloch)

One medium-sized specimen was injected with 0.15 cc. of adrenalin 1:1000. Fifteen

seconds after injection a very red pattern appeared in the caudal and dorsal fins, undoubtedly caused by the dispersion of pigment in the erythrophores. The lateral yellow stripes also became very pronounced.

After five minutes there was some lightening of the iris about the periphery of the pupil. This species is mostly white, so it was a little difficult to determine just how much effect the adrenalin had.

Twenty minutes after injection the dorsal surface of the fish was still a faint green. The iris also remained a faint green. Neither at this time nor at any subsequent time was there any sign of erasure of the black or yellow lateral stripes. The fish was found dead on the following morning.

One individual was injected with 0.25 cc. of intermedin (Choay, 2000 units per cubic centimeter). The standard length was 148 mm., and the weight 61 grams. Ten minutes after injection the fish was in a "plaid" phase, with rather strong stripes and bars. The pink markings were prominent. However, this is the phase that the fish usually maintained in the laboratory aquarium. There was no change during five hours of observation.

One *Iridio* weighing 12.3 grams and measuring 92 mm. in standard length was injected with 3 milligrams of melanophore-dispersing hormone. Three minutes after injection there appeared to be some dispersion of pigment in erythrophores over the dorsal region. The black lateral stripes were prominent. Compared with the non-injected fish on the same background, the experimental fish showed a difference only in increased red coloration. This coloration lasted for approximately two hours; then the normal pattern was gradually reassumed.

##### FAMILY SCARIDAE

###### *Sparisoma abildgaardi* (Bloch)

One specimen weighing 4440 grams and measuring 260 mm. in standard length was injected with 1.6 cc. of adrenalin 1:1000. The fish was in a dark phase before injection and maintained the dark phase throughout the observation period. Four minutes after injection the iris began to whiten around the periphery, and some of the individual scales lightened, giving a freckled appearance. Seven minutes after injection light patches

appeared along the dorsal surface of the fish, and after 10 minutes these light patches were seen over the entire body, particularly around the site of injection. The iris at this time was entirely opaque white. One-half hour after injection the coloration had not changed. It was interesting to note that the top of the eyeball, which is devoid of scales and is exposed somewhat in this species, retained its color and was unaffected by the adrenalin. One hour and twenty minutes after injection the iris was still light, but some color had returned. After two hours the iris was back to its normal coloration. Some light patches were still seen on the lateral surfaces, but otherwise the dark phase was still maintained.

Four hours after injection the coloration of the animal was entirely normal, exactly the same as that of a non-injected control.

#### ORDER CHROMIDES

##### FAMILY POMACENTRIDAE

##### *Pomacentrus leucostictus* (Müller and Troschel)

Two fishes were injected. One measuring 77 mm. in standard length and weighing 20 grams was given 0.075 cc. of adrenalin 1:1000. The other, smaller, fish measuring 67 mm. in standard length and weighing 12 grams was given 0.1 cc. of adrenalin 1:1000. Both fishes were adults and were dark in color, with lateral vertical dark bars and yellow pectoral fins. Immediately after injection the scales about the point of insertion of the needle became white. After two and one-half minutes the white area had spread somewhat, making a small whitish area. After five minutes the iris had begun to lighten, particularly about the periphery. Twenty minutes after injection the irises of both fishes were conspicuously light, with a few dark patches. No further pigmentation change was noted in the smaller fish, which had received the larger dose of adrenalin. The rest of the body remained in an extremely dark phase. Thirty-seven minutes after injection both fishes showed normal coloration of the iris. The larger fish, which had received the smaller injection, showed a peculiar reaction that lasted about three hours and that began about two hours after the injection was made. The surface of the body covering the region

of the body cavity became white. This area extended from immediately posterior to the insertion of the pectoral fins to the vent. The dorsal part of the area appeared to be delineated by the dorsal part of the abdominal cavity. The fish appeared in good condition and was active and rather pugnacious towards the non-injected control.

Under the dissecting microscope the pigment granules in the melanophores of the white area were seen to be concentrated to points. The xanthophore pigment granules of the pectoral fins were fully dispersed. The line of demarcation of the white area from the rest of the dark body was not so sharp when seen under the microscope as when observed grossly, but was formed by a gradual though rapid transition from concentrated to dispersed melanophores.

This species is not one that ordinarily changes color rapidly. It is obvious that the adrenalin did not act on the nervous system to produce concentration of the pigment in dermal melanophores. The white area was probably caused by local action, the adrenalin dispersing through the tissues surrounding the body cavity into which the injection was made.

##### *Abudefduf saxatilis* (Linnaeus)

One specimen weighing 70 grams and measuring 113 mm. in standard length was injected with 0.15 cc. of adrenalin 1:1000. This fish had been living in an outdoor pool for five or six weeks. When caught and put in a small tank for easier observation, the coloration became extremely dark. All the yellow disappeared, and the light bands became nearly as dark as the black bands. Some blue color could still be seen over the dorsal region. The fish was still in this intense dark color four hours later when it was injected, although the observation tank was provided with a white sand bottom.

One minute after injection the iris became white. The light bands of the pattern lightened and the dark bands darkened a little immediately after the fish had been handled, but subsequently the fish resumed the all-over dark phase. Five minutes after injection white patches appeared around the mouth, on the lower jaw, and on the head anterior to the eyes. Eight minutes after injection irregu-

lar white patches were seen over the entire body. In many places this reaction was confined to the dermal chromatophores, the pigment granules of those in the scales remaining dispersed. A white background was thus seen through a black scale overlay. After 15 minutes the edge of the caudal fin for about one-eighth of an inch from the tips showed lighter than the rest of the fin, although not white.

After 45 minutes the fish was observed under the low power of the dissecting microscope. Most of the scales showed dispersed melanophores. The dermal melanophores were seen to be concentrated to pinpoints in the white patches, and the iridophores were very prominent. Xanthophores were all in a dispersed state. In places where the black band of the pattern was found, no very white tissue was seen beneath the scales. The fish appeared to be dying for a few seconds after this handling but then righted itself, and for the first time since it was removed to the observation tank exhibited the normal bold, barred pattern. Four hours after injection the coloration including that of the iris was back to normal.

#### ORDER OPHIDIOIDEA

##### FAMILY CARAPIDAE

##### *Carapus bermudensis* (Jones)

One specimen was injected with 0.025 cc. of adrenalin 1:1000. After one minute the melanophores in the meninges and in the external coat of the eyeball had contracted, leaving brain and eyeballs bluish white with the effect produced on the iridophores. After two minutes the melanophores of the peritoneum showed concentration of pigment and the abdominal cavity became outlined with silver as a result of the concomitant dispersion of crystals in the iridophores. Three minutes after injection, the melanophores about the spinal cord showed concentration of granules, although the scarce dermal melanophores were still in a rather dispersed condition. After four minutes the snout still remained dark, but the color of the band of black at the base of the skull was diminishing. Melanophores along the dorsal surface had concentrated pigment. The iris was completely white and the pupil appeared as a mere slit. Erythrophores everywhere still

showed dispersed pigment. Six minutes after injection the melanophores over the snout and over the insertion of the body muscles at the base of the skull still imparted a darker appearance to these areas but only because of their greater numbers in these regions.

The extreme transparency of the tissues of this species makes it an excellent one for study of the reactions of the internal melanophores. Their greater sensitivity to adrenalin is noted at once, for both dermal and internal chromatophores can be observed simultaneously and the slower reactivity of the dermal melanophores is obvious.

#### ORDER GOBIOIDEA

##### FAMILY ELEOTRIDAE

##### *Erotelis smaragdus* (Cuvier and Valenciennes)

One specimen weighing approximately 5 grams and measuring 99 mm. in standard length was injected with 0.05 cc. of adrenalin 1:1000. The fish was in a dark brown phase when injected, although the tank in which it had been kept had a light sand bottom and contained no dark-colored fittings. Three seconds after injection the animal paled all over except for the tips of the caudal and anal fins. Two minutes and 20 seconds after injection the dorsal fin was colorless, although the tip of the caudal was still dark. Six and one-half minutes after injection the whole fish was extremely pale, including the tip of the caudal. The vascularization of the body musculature was visible to the naked eye. This species apparently has few if any iridophores or leucophores; thus a translucent effect of the skin was produced by the reaction of the chromatophores to adrenalin rather than the opaque white seen in *Bathygobius*. After two hours the pigmentation had returned to the caudal fin and after six hours the fish was all dark again except for a few light patches on the dorsal surface.

##### FAMILY GOBIIDAE

##### *Bathygobius soporator* (Cuvier and Valenciennes)

Four individuals of this species were injected, each with 0.05 cc. of adrenalin 1:1000. The reaction was quick in all fishes. The pigment granules within the melanophores became concentrated almost at once, leaving a

pattern made by the xanthophores. Finally the pigment granules in the xanthophores became concentrated, leaving the fishes almost pure white. About two hours after the first fish was injected, the normal coloration, and in this case the dark phase, had returned over the entire body posterior to the head. A patch on the forward part of the head including the region of the eyes remained white, so that the animal seemed to be wearing a white mask. The next morning the coloration appeared entirely normal. The fish appeared in good health and ate well during the afternoon, but nevertheless was found dead the following morning.

The time reactions of the second injected fish were as follows: One minute after injection the animal appeared pale but still showed some pattern of melanophores. Five minutes after injection the gray color had completely faded, and the yellow of the xanthophores predominated in the pattern. The iris was completely white. Eight minutes after injection the photograph represented by plate 10, figure 2, was taken. The light fish is the injected animal, and the pattern that shows was made mostly by xanthophores. Twenty-eight minutes after injection the pattern appeared a little more distinct, with a darkening of the edges of the dorsal and caudal fins. However, three hours after injection the animal was still white. Five hours after injection the gray color had completely returned, except for the white patch on the head between the eyes. This light patch was still prominent one hour later when the rest of the fish was in an extremely dark phase, much darker than the non-injected control.

The control animal photographed measured 60 mm. in standard length, and the injected fish measured 58 mm.

The third and fourth gobies were injected at approximately the same time, and one was put in the dark room with its control while the other remained in the light laboratory. Both injected fishes lightened immediately, and there was no difference in subsequent reactions shown by the fish in the light from that in the dark. Lights were turned on in the dark room one-half hour after injection, and the injected fish was found to be very white, while the non-injected control was found to be in a very dark phase. The dark phase returned to the injected animals about 65 min-

utes after the injection had been made except for the white face "mask."

One individual was injected with 0.20 cc. intermedin (Choay, 2000 units per cubic centimeter). Ten minutes after injection the fish appeared a little darker but definitely more yellow, and the black bands across the dorsal surface appeared more pronounced. The fish was kept throughout the observation period on a white sand bottom. Twenty-five minutes after injection the fish appeared no darker but definitely yellower. The xanthophores appeared to be affected more than the melanophores. The fish maintained this darkish yellow pigmentation up to seven hours after injection, when the observation period ended. The following morning the fish was in a more normal, light gray phase. The same fish was injected again with the same dosage and studied under the dissecting microscope. The dark yellow phase appeared within 10 minutes after injection. Xanthophores were fully dispersed. Melanophores were in the fully dispersed condition in the black bands but were punctate in the light bands.

One large goby weighing 16.6 grams and measuring 88 mm. in standard length was injected with 4 milligrams of melanophore-dispersing hormone. Two minutes after injection the fish appeared in a dark phase with a yellow color over all. While dark, the injected fish was lighter in color than its non-injected control. Fifteen minutes after injection the fish showed a dark orange-yellow color, and the black pattern had begun to fade. Forty-five minutes after injection the black pattern had faded still more, and the fish appeared a dull reddish orange. Three hours later the black bands of the pattern had returned, but the light areas retained the red-orange coloration. This species responds in this way to many other mammalian pituitary preparations. Twenty-four hours later the fish still maintained a slight yellowish cast.

#### ORDER HAPLODOCI

#### FAMILY BATRACHOIDIDAE

#### *Opsanus* sp.

Two toadfishes were injected, each with 0.05 cc. of adrenalin 1:1000. The reaction in these fishes was much the same as in the goby. The first whitened within two minutes. Seventeen minutes after injection the iris was still

dark except for two small white spots, one on the dorsal and one on the ventral aspect, making a vertical stripe through the eye. Four hours after injection the fish was still white, but at the end of five hours there was some semblance of the normal coloration (pl. 10, fig. 3). Seven and one-half hours after injection the normal coloration had completely returned.

The second injected toadfish showed the same reactions. Several minutes after injection the inside of the mouth was noted to be still black, although the outside of the animal had already become white. Gradually the mouth lost its blackness also but never became any lighter than a light gray.

#### ORDER PLECTOGNATHI

##### FAMILY BALISTIDAE

##### *Balistes capriscus* (Gmelin)

One specimen weighing 148 grams and measuring 141 mm. in standard length was injected with 0.3 cc. of adrenalin 1:1000.

The iris began to lighten after 45 seconds, and there was an opaque white spot at the site of the injection. The pattern on the body was pronounced. Two minutes after injection the iris was all white, and the pronounced pattern of the body was unchanged. Five minutes after injection the lips and a small area about the mouth appeared light. The membrane behind the trigger was also white. The coloration of the rest of the fish was unaffected. Two and one-half hours after injection the fish appeared as it had before injection, with all color returned to iris, trigger membrane, lips, and injection site.

##### FAMILY MONACANTHIDAE

##### *Monacanthus ciliatus* (Mitchill)

One individual was injected with 0.05 cc. of intermedin (Choay, 4000 units per cubic centimeter). The standard length was 56 mm. and the weight 5.2 grams. At the time of injection the fish was in a grayish phase, neither so light nor so dark as it was capable of becoming. Throughout a four-hour observation

period after injection there was no change whatever in the coloration.

One individual weighing 6 grams was injected with 2 milligrams of melanophore-stimulating hormone (Armour) dissolved in 0.06 cc. of saline solution. The fish was in a rather black, white, and gray color phase when injected. The color remained unchanged for more than one-half hour; then a faint greenish tinge appeared over the anterior and dorsal parts of the body. No other reaction was noted, and the fish did not become nearly so dark as it is normally capable of becoming under proper stimulation.

#### ORDER PEDICULATI

##### FAMILY OGCOCEPHALIDAE

##### *Ogcocephalus radiatus* (Mitchill)

One individual of this species was injected on two successive days. The standard length was 135 mm., and the weight was 74 grams. The first day it received 0.1 cc. of adrenalin, a quantity that was insufficient to cause much reaction. Ten minutes after this injection the iris lightened somewhat, and after 13 minutes the light band across the caudal fin and the pectoral fins had also lightened. Twenty minutes after injection the pattern of the iris was nearly obliterated. Before injection the over-all coloration of the body had been a mottled brown and tan, and neither pattern nor intensity of coloration was changed by this injection.

The following day the same fish received an injection of 0.2 cc. of adrenalin. After two minutes the pigmentation of the iris was noticeably reduced. After four minutes the all-over coloration of the fish was darker than before injection, but the light band of the caudal and pectoral fins was more pronounced. The fish was quite active for this species, swimming around the tank in which it usually rested quietly on the bottom. Ten minutes after injection the ventral fin membranes were white, and some white patches were seen on the dorsal and lateral surfaces as far posteriad as the caudal peduncle.

#### RESULTS OF ADRENALIN AND INTERMEDIN ADMINISTRATION

Results of these injections are summarized in table 2. Several interesting facts emerge from a study of the table and the detailed

notes. The action of adrenalin is not uniform in teleosts. In some species in which all the melanophores react to the hormone by con-

centration of their pigment granules, adrenalin appears to act upon the nervous system. Fishes such as *Amieurus*, *Carapus*, and *Bathygobius*, show a prompt reaction of all the melanophores regardless of their morphological position. In other instances, adrenalin causes the concentration of melanin granules only in those melanophores that are not dermal or epidermal in their distribution, particularly the perineural melanophores. The perineural reaction is usually detected in the intact fish by the reaction in the iris. Only in a very translucent species such as *Carapus* can this reaction be seen in other places without dissection. Perineural melanophores react more quickly and more uniformly than do dermal or epidermal ones.

In phylogenetic terms, it is interesting to note that the melanophores of the fishes of the less specialized orders are most responsive to adrenalin. More advanced orders of fishes show a response to the hormone in perineural melanophores only. Even this reaction is not observed in *Angelichthys*, *Pomacanthus*, and *Acanthurus*. Then response to adrenalin again appears in fishes more specialized than the last three mentioned; first it is seen in the perineural cells and finally seen in all the melanophores of *Carapus*, *Bathygobius*, and *Opsanus*. *Epinephalus* is also responsive to adrenalin. Among the species used herein, all the acanthopterygians found to respond to adrenalin administration were fishes of bottom-living habits. All the spiny-rayed fishes inhabiting the surface of mid-water areas were unresponsive to adrenalin. Whether or not this has any particular biological significance is unknown. Perhaps this difference in surface- and bottom-dwelling forms is related to the ready accessibility of places to hide for the latter. The faster nervous control of coloration may be more important to fishes that cannot find shelter quickly.

Adrenalin obviously can cause concentration of melanin granules when directly applied to scales, even in those fishes in which the hormone is ineffective when carried by the blood stream. Some of the peculiar freckling effects of the skin, described in various species above, are probably due to a contamination, by small amounts of the hormone, of the nets or the area on which the fishes were placed for injection. Direct effect of adrenalin on melanophores is the cause of

the depigmented region around the site of injection in fishes which otherwise show no pigmentary response to adrenalin. The depigmented region of the body cavity in *Pomacanthus* was obviously caused by the action of the adrenalin, which had been injected intraperitoneally, directly on the peritoneal melanophores and on the dermal melanophores through the body wall.

In most cases the lipophores, xanthophores, and erythrophores responded to adrenalin by dispersion of the pigment granules within the cells. This response is at times only a partial one, and at times it is a secondary response after an initial concentration of pigment. Two exceptions are *Bathygobius* and *Opsanus* in which the xanthophores are concentrated by adrenalin. Attention is called here to the fact that adrenalin must be fresh if comparable results are to be obtained. Thus *Bathygobius*, when injected with fresh adrenalin, responds by concentration of both melanophores and xanthophores and by dispersion of leucophores. This reaction results in an opaque white over-all color. When adrenalin that has become discolored with age is used, only the melanophores become concentrated, and the xanthophores and leucophores are dispersed, resulting in an over-all color of clear, bright yellow.

Breder and Rasquin (1955) described the reactions of *Chaetodipterus faber* after injection of adrenalin. Perineural melanophores were concentrated as shown by the change in the coloration of the iris. The over-all color of the fish, however, was much darker than that of the control; dermal melanophores were therefore in a dispersed condition. It is believed that the dermal reaction was not a direct effect of adrenalin, but a result of handling or an extraneous visual factor in a fish in which the dermal melanophores were not affected by adrenalin. The administration of adrenalin to other species reported herein cannot be said actually to cause dispersion of melanin granules. Among fishes closely related to *Chaetodipterus*, in which melanophores were not affected by adrenalin, there was also no dispersion of pigment granules in lipophores or leucophores. The reaction of teleost chromatophores of any type to adrenalin is therefore not consistent.

The reaction of xanthophores and erythrophores to injection of intermedin is a con-



sistent one. These chromatophores disperse their pigment granules under the influence of this pituitary hormone. Only certain species, however, show a response of melanophores to intermedin. Among the fishes used here dispersion of melanin granules was confined to *Ameiurus*, *Gambusia*, *Astyanax*, and *Atherina*. No results of the hormone administration were seen in melanophores of the other spe-

cies tested. Leucophores appeared to be unaffected by intermedin.

Therefore, depending upon the species and probably also the dosage, adrenalin can cause concentration of melanophores, either concentration or dispersion of lipophores, and dispersion of leucophores, or it may be non-reactive.

#### ACTION OF MAMMALIAN PITUITARY HORMONES ON PIGMENTATION OF *ASTYANAX* AND *BATHYGOBIUS*

Seven anterior pituitary preparations were tested on the fresh-water characin *Astyanax mexicanus*; eight anterior pituitary preparations were tested on the marine gobiid *Bathygobius soporator*. The hormones used, the numbers of fishes tested, and the dosages are found in table 4. The hormones were dissolved or suspended in 0.6 per cent saline solution and administered intraperitoneally. The fishes were measured, weighed, and injected at the same time under light urethane anesthesia. The controls were injected with similar volumes of 0.6 per cent saline solution. Because some of these hormones contained various amounts of oxytocin and vasopressin, a control group of *Bathygobius* were injected with purified oxytocin (Armour lot P12602).

A second control group received injections of vasopressin (Parke, Davis, and Co., pitresin). Moreover, because the yellow pigmentation of some amphibians appears at metamorphosis when the thyroid is most active (Twitty and Bodenstein, 1939; Stearner, 1946), a group of *Bathygobius* was injected with thyroxin, as it was conceivable that the thyroid might have been stimulated by any or all of these mammalian hormones.

Table 5 gives a list of fishes that were given doses of MSH in quantities of approximately 1 milligram per 4 grams of body weight. Doses of this order of magnitude were shown by Chavin (1956) to be sufficiently potent to cause melanin dispersion in goldfish.

Oxytocin and vasopressin had no effect on

TABLE 4  
COLLECTED DATA AND RESULTS OF VARIOUS HORMONE INJECTIONS ON PIGMENTATION

| Species and Hormone Injected            | No. of Fishes | Dosage                  | Pigmentation           |
|---|---------------|-------------------------|------------------------|
| <i>Bathygobius soporator</i> (marine)   |               |                         |                        |
| Growth (Somar lot M208)                 | 4             | 1 mg./g. weight         | Xanthophores dispersed |
| Prolactin lot 759-014-A                 | 4             | 1 mg./g. weight         | Xanthophores dispersed |
| TSH lot PRR 3-128-92                    | 8             | 1 mg./g. weight         | Xanthophores dispersed |
| TSH lot 317-51                          | 4             | 1 mg./g. weight         | Xanthophores dispersed |
| MSH lot D216-155-C                      | 4             | 1 mg./g. weight         | Xanthophores dispersed |
| FSH lot R377201                         | 4             | 1 mg./g. weight         | Xanthophores dispersed |
| LH lot R377242 H                        | 8             | 1 mg./g. weight         | Xanthophores dispersed |
| ACTH lot 212-103                        | 4             | 1 mg./g. weight         | Xanthophores dispersed |
| Thyroxin lot 22227                      | 4             | 2 mg./g. weight         | No reaction            |
| Oxytocin lot P12602                     | 4             | $\frac{1}{2}$ unit/fish | No reaction            |
| Vasopressin                             | 4             | 1 pressor unit/fish     | No reaction            |
| <i>Astyanax mexicanus</i> (fresh water) |               |                         |                        |
| Growth (Somar lot M208)                 | 4             | 1 mg./g. weight         | No reaction            |
| Prolactin lot 759-014-A                 | 4             | 1 mg./g. weight         | No reaction            |
| TSH lot 317-51                          | 4             | 1 mg./g. weight         | No reaction            |
| MSH lot D216-155-C                      | 4             | 1 mg./g. weight         | Melanophores dispersed |
| FSH lot R377201                         | 4             | 1 mg./g. weight         | No reaction            |
| LH lot R377242 H                        | 4             | 1 mg./g. weight         | No reaction            |

TABLE 5  
RESULTS OF ADMINISTRATION OF MSH TO VARIOUS FISHES AT A DOSAGE LEVEL OF 1  
MILLIGRAM PER 4 GRAMS OF BODY WEIGHT

| Species                     | Pigmentation                            |
|-----------------------------|---|
| Fresh water                 |   |
| <i>Ameiurus nebulosus</i>   | Melanophores dispersed                  |
| Euryhaline (in sea water)   |   |
| <i>Gambusia</i> sp.         | Melanophores and xanthophores dispersed |
| <i>Cyprinodon baconii</i>   | No reaction                             |
| Marine                      |   |
| <i>Atherina stipes</i>      | Melanophores dispersed                  |
| <i>Lutianus apodus</i>      | Xanthophores dispersed                  |
| <i>Irideo bivitatta</i>     | Erythrophores dispersed                 |
| <i>Monacanthus ciliatus</i> | No reaction                             |
| <i>Chaetodon striatus</i>   | Xanthophores dispersed                  |

the pigmentation of any of the fishes. MSH was the only hormone that caused a change in pigmentation in fresh-water fishes. In *Astyanax*, the melanophores became dispersed especially in the fins, but the fishes did not assume so dark a coloration as they normally take on when living over a dark bottom. The catfish, *Ameiurus*, assumed a dark coloration within a few minutes after injection of MSH, and the coloration deepened until the fish appeared completely black except for the ventral region. This extremely dark phase lasted for several hours, and normal coloration did not return until the following day.

The marine *Bathygobius* assumed a yellow coloration after injection of all the pituitary hormones except those of the posterior lobe. The yellow color of the gobies was caused by the dispersion of pigment granules in the xanthophores. TSH, FSH, and LH were most effective, and prolactin and ACTH least effective, in bringing about the color change, with the effectiveness of growth hormone and MSH falling between the two extremes. The melanophores did not appear to be changed in any way. The fish were kept over a white coral sand ground and before injection displayed various patterns of bold black and white, or gray. These individual patterns remained the same after injection except for the addition of the yellow color. The time required for the reaction to take place varied.

Most fishes required from 10 to 20 minutes to show the yellow color. However, after injection of TSH lot PRR-3-128-92 the change occurred in two minutes. The effects were slow to wear off; frequently the fishes did not return to normal coloration for several days.

Only two species in salt water responded to MSH injection by some dispersion of melanin granules, namely, *Atherina* and *Gambusia*. Of these, *Gambusia* is a euryhaline species which can be readily transferred directly from fresh to salt water and vice versa without any ill effects. In the case of *Atherina* the melanophores around the mouth and chin and in the lateral rows showed partial dispersion. The *Gambusia* responded to MSH first by dispersion of pigment granules in xanthophores in the meninges. Later, dermal melanophores were in a dispersed condition over the entire body and in the fins, and the injected fish displayed a notably more yellow coloration than the non-injected control owing to the dispersion of dermal xanthophores at the same time. All other marine fishes listed in table 5 responded only by dispersion of xanthophores and erythrophores; melanophores were not altered in any way. Six of the total number of injected fishes died within 48 hours after the injection. It is believed that the deaths were due not to any toxic properties of the hormones but rather to lesions created by the hypodermic needle in vital organs.

## DISCUSSION

### PART I

THERE IS GENERAL AGREEMENT among investigators that the pineal organ in all fishes, including cyclostomes and elasmobranchs, is made up of three types of cells, namely, sensory cells, supporting cells, and ganglion cells. Tretjakoff (1915) working with *Petromyzon*, and Holmgren working with *Squalus* (1918a) and teleosts (1920), reported that neurons lead off from the basal ends of the sensory cells and combine with the ganglion cells. Axons from the ganglion cells then run through the pineal stalk to the posterior commissure and midbrain. These conclusions were based on studies of tissues stained with methylene blue. Both these investigators described the sensory cells as having cytoplasmic projections into the lumen of the gland. Holmgren (1920) conceived a cycle of apocrine secretion for these cells consisting of growth of the projecting process ("Spitzenstück"), accumulation and liquefaction of mitochondria within it, and subsequent rupture of the projection, freeing the secretion into the lumen. Tretjakoff (1915) believed that secretion was elaborated by the supporting cells in *Petromyzon* rather than by the sensory cells. Galeotti (1896, cited in Tilney and Warren, 1919) observed fuchsinophile granules in the pineal cells of *Leuciscus* which he interpreted as evidence of secretory activity, and apparently he was the first to suggest that the secretion product was delivered to the ventricle of the dienkephalon.

Friedrich-Frekxa (1932) considered that his work on the teleosts *Arius*, *Plotosus*, *Salmo*, *Xiphophorus*, *Gobius*, *Hemiramphys*, and *Dermogenys* confirmed Holmgren's thesis of secretory function of the sensory cells. Using *Dermogenys* for detailed histological examination, he could find no evidence of secretion in supporting cells; after Susa fixation and iron hematoxylin and acid fuchsin staining, the cytoplasm of the supporting cells remained unstained. In other species the histological distinction between sensory and supporting cells was not so apparent, and the author considers the possibility that a type of glandular epithelium may be formed in

which both types of cells are involved and have similar potencies. In *Dermogenys*, Friedrich-Frekxa was able to demonstrate, by azan staining, a blue cap or outer portion of the sensory cell process as distinguished from the violet-stained main portion of the cell. The blue caps often had a granular appearance, and blue granulation was also found in the lumen into which the sensory cell processes projected. He considered this to be a confirmation of apocrine secretion by these cells. The same stained granulation was also noted in capillaries. Because he could distinguish secretion-filled capillaries and cell-filled capillaries, he advanced the theory that secretion was stored in some capillaries, confined there by the constricting action of Rouget cells in the capillary walls. The secretion would be released to blood vessels of the chorioid plexus by means of an increase in blood pressure. In as much as the author assumed that the pineal regulates fluid pressure, he deduced therefore that the secretion is a hormone that influences the diameter of the blood vessels or the permeability of the chorioid membrane to cause a lowering of the fluid pressure. In the opinion of this author, then, the function of the pineal organ is to lower hydrostatic pressure.

The idea that the pineal organ has some function connected with hydrostatic pressure is still considered. Van de Kamer (1952) believes that the best theory of the function of the organ is that it is sensory rather than glandular in character and that the sensory function is to measure the pressure of cerebral fluid or to sense its chemical composition. This investigator cites as evidence the lumen of the organ found in fishes, amphibians, reptiles, and birds which communicates with the brain cavity. However, he makes the assumption that epiphysis and paraphysis are homologous.

The manner in which the pineal organ functions in teleosts is still undefined. Von Frisch (1911b) stated that sensory cells are not found in the walls of the pineal, although the organ is connected by nerve fibers to the brain. He thought that the organ was glan-

dular in spite of his work with *Phoxinus* showing a light-receptive function in the epiphysis of that species. On the other hand Studnicka (1905), who made very careful studies of teleost pineals, claimed that there is no evidence to show that the cells that make up the organ are entirely glandular, nor is any secretion product found within the lumen.

Modern histochemical methods have been used by Wislocki and Dempsey (1948) on the pineal gland of the rhesus monkey, and the results show that the gland is neither atrophic nor rudimentary. In this animal the organ is entirely glandular in character, and the parenchyma is made up mainly of fairly uniform epithelioid cells. Application of the Bauer-Feulgen stain gave negative results, but the use of McManus' periodic acid technique produced a red staining in the cytoplasm of some of the cells. This reaction was prevented by previous exposure of the sections to saliva, which indicates that the positive reaction was the result of the presence of glycogen. The authors consider the periodic acid test a more delicate one than other tests for the demonstration of glycogen and have concluded that the pineal cells contain very small amounts of the substance not demonstrable by other techniques. The use of Bodian's protargol method demonstrates fine nerve fibers associated mainly with the reticular fibers. Sheaths or networks of reticular fibers were found to surround the blood vessels. Parenchymal cells, blood vessels, and the perivascular sheaths gave positive reactions to tests for alkaline phosphatase. The parenchymal cells were also found to contain ribonucleoprotein, and the authors suggest that such cytoplasm combined with the large, chromatin rich nuclei indicates active nucleoprotein synthesis by the cells.

Further histochemical examinations of the pineal of the rat were made by Leduc and Wislocki (1952). Parenchymal cells showed a strong positive reaction for acid phosphatase, present mainly in the nuclei. A moderate positive reaction for non-specific esterase was shown in frozen sections of the pineal, and a moderate positive reaction was also shown for succinic dehydrogenase. The authors conclude that the results are indicative of marked metabolic activity but not necessarily of secretion. They consider the

results to be compatible with the possibility of neurosecretion. This last consideration is difficult to understand unless the parenchymal cells are thought to be nerve cells. Other neurosecretory cells abound in granulations characteristic of secretory cells.

That the mammalian pineal is a gland with endocrinological function is therefore still disputed, and its exact role is not well understood. McCord and Allen (1917) indicated that desiccated pineal powder had some ability to influence the pigmentation of amphibians. Melanin granules were concentrated in all the dermal melanophores of tadpoles of *Rana pipiens*, *Rana cantabrigiensis*, and *Bufo americanus* within 30 minutes after the ingestion of desiccated pineal. Furthermore, the authors found that the pigment-concentrating factor was completely dissolved in acetone. Positive results were obtained by the use of acetone extracts, but no results were obtained when the residue from acetone extraction was used. Pineal extracts had no effect on the pigmentation of the adult *Fundulus* but produced concentration of melanin granules in larval, one-week old fish. The implantation of *Atherina* pineals into *Cyprinodon*, which was done for behavior experiments for the present report, had no observable effects on the pigmentation of the host fish. Pigmentation changes associated with pineal activity in teleosts are probably not dependent on any hormone released by the pineal. Rather, it seems more likely that the pineal activity is regulated in some way by the pigmentary changes that admit light to or exclude it from the organ.

Simmonet, Thiéblot, and Segal (1952) also showed the concentration of melanin in melanophores after treatment with pineal extracts in the frog *Rana esculenta*. In hypophysectomized frogs the pineal extracts had no effect on the already concentrated melanin in the chromatophores. The opposite effect, that of dispersion of melanin in the melanophores, occurred in the same animals after pinealectomy.

For some time sexual anomalies have been known to accompany pineal pathology in human beings. Recent experimental evidence has shown that a definite relationship exists between pineal and gonads in birds and mammals, particularly in the immature forms or juvenile forms. Fischer (1943) published a

technique for extraction of pineal glands, and the biological assay for this material was based on the inhibition of the opening of the vaginal membrane in immature female mice. Simmonet, Thiéblot, and Melik (1951) found that pinealectomy in young rats increased the weight of the ovaries, increased the numbers of corpora lutea, and accelerated the vaginal changes associated with sexual maturation. Simmonet and Sternberg (1951) have reported acceleration of sexual maturation and increased total phosphorus content in the testes of rats after pinealectomy. Dogs also have been reported to respond to pinealectomy with accelerated sexual development (Simmonet and Thiéblot, 1951).

Shellabarger (1953) found hypertrophy of the testes in pinealectomized cockerels if sufficient time were allowed to elapse between operation and autopsy (40–60 days). Increase in testis weight of pinealectomized chicks could be prevented by the injection of mammalian pineal material. Increased androgen secretion was shown in pinealectomized birds by increased size of the comb. Some interaction of pineal and pituitary was suggested because in some cases the pituitaries of pinealectomized birds showed increased gonadotrophic potency over those of the controls. Gonadotrophic potency decreased following the administration of pineal material. However, the pituitary involvement does not necessarily depend on a direct association with the pineal, as pituitary changes could be the result of changes in the gonads. This investigator reported that histological examination of the testes failed to show any quantitative differences after pineal extirpation or injection. This is difficult to understand, as the weights of the organs were definitely influenced. Qualitative differences were not discussed.

Moszowska (1947) has shown an antagonism between epiphyseal and hypophysial tissues *in vitro*, the embryonic epiphyseal tissue being capable of diminishing and even annulling the gonad-stimulating effect of the hypophysial tissue on the male gonad. Here again, the chicken was used to provide the experimental tissue, and there seems no doubt that in the fowl the epiphysis has a glandular function.

The action of the pineal on the pituitary is believed to be a direct one by Thiéblot

(1954). In this latest paper he has reviewed many reports dealing with the effects of pinealectomy on the pituitary and its target organs, the gonads and the adrenals, in mammals and birds. In immature rats there is impressive evidence of an association between pineal and pituitary, for pinealectomy is followed by increase in volume and weight of the gonads, both male and female organs, and in the ovary by an increase in numbers of corpora lutea. These glands in turn function to increase the weight of the seminal vesicles in the male, and to cause premature rupture of the vaginal opening in the female. In the adrenal of pinealectomized male rats the proportions of cholesterol and ascorbic acid are distinctly lowered, while in the female neither of these is modified. The author believes the positive results in the male to be associated with the enhanced secretion of testosterone. Changes in the cellular content of the rat pituitary after pinealectomy were slight. Increase in per cent of acidophiles was approximately 10 per cent but the increase in basophiles only 0.9 per cent. This slightly augmented number of basophiles, then, is presumably responsible for the increased gonadotropic potency of the hypophysis. Administration of pineal extracts and implantation of pineal glands were observed to have inhibitory effects on the development of the gonads. This author is convinced that the action of the pineal is hormonal and exerts an inhibitory action on the gonadotropic activity of the hypophysis. Simmonet, Thiéblot, Melik, and Segal (1953) considered the LH and LTH factors to be the ones particularly involved because of the modifications of the female genital tract.

Grunewald-Lowenstein (1952) has also attempted to relate the action of the pineal to that of the pituitary but in a totally different way. Using the pupillary reactions to light stimuli of the pigeon's eye, she recorded the results of pineal and pituitary implantation. Considering that the parasympathetic nervous system was responsible for the contraction of the pupil and the sympathetic system for the dilation, she found that pineal implantation increased the activity of the sympathetic and implantation of anterior pituitary increased the activity of the parasympathetic. The author suggests that sympathetic control of various bodily functions

is stimulated by the pineal and that this is the reason behind the conflicting results other investigators have reported for pineal function. Such stimulated functions include thermoregulatory processes, cardiovascular phenomena, and metabolic changes in blood calcium and blood sugar, predominantly controlled by sympathetic activity. The author even relates the precocious sexual development of the young human male observed to follow pinealectomy or to accompany the presence of pineal tumors to the presence of abundant sympathetic nerve fibers in the testis, and to the absence of similar innervation to the secretory tissue of the ovary. This is an interesting and ingenious theory which needs confirmation in many respects.

From cytological study of pineal organs of various teleost species it is obvious that all have certain common morphological features. Those that are highly developed, as in *Atherina* and *Jenkinsia*, are formed of the same cellular components as those that appear to be nearly rudimentary, as in *Hippocampus* and *Monocanthus*. The increased development is only a matter of increase in size of the organ compared with other parts of the brain and increase in surface caused by invaginations and convolutions of the walls of the vesicle.

The organ in teleosts appears to have either a dual function or achieves a single function by a dual control. The behavior of fishes in response to light is certainly affected by the pineal or the pineal region, and those species in which the pineal is most highly developed have the organ so placed that it is extremely accessible to the impingement of light. The connection between free nerve endings and fibers that enter the habenular commissure also argues on the side of a sensory function for the organ.

That the pineal in teleosts has also a secretory function is no longer open to doubt. Regardless of the opinions of early investigators who declared that no secretion product could be found in the lumen, cytological and histochemical methods have shown that there is a secretion present and that it is probably an apocrine one. An apocrine secretion has already been postulated by Holmgren (1918a, 1918b, 1920) for elasmobranchs and teleosts and by Friedrich-Freksa (1932) and Grune-

wald-Lowenstein (1956) for teleosts. The secretion contains both glycogen and glycoproteins, in as much as stains for these two constituents do not stain the same areas in section. Possibly those fishes in which the pineal areas are exposed to light have a cycle of glycogen production or storage, as an increase of glycogen was noted to occur in *Atherina* pineals during the hours of darkness.

The presence of a parietal foramen in the skull does not necessarily imply the presence of a particularly specialized pineal organ. In some species with very specialized pineals, as *Atherina stipes* and *Jenkinsia lamprotaenia*, the tissues overlying the pineal area, which include cartilage, bone, connective tissue, bony scales, and epithelium, are so completely transparent that a veritable window is formed through which light falls on the pineal area and whatever adjacent areas of the brain are thus exposed. In *Astyanax*, which is provided with a foramen in the skull over the pineal area, the pineal organ itself is not particularly large or specialized. The same observation can be made of the tuna and the bonito which are obviously provided with an elaborate specialization of tissues overlying the pineal area, including a parietal foramen to allow for the penetration of light. Yet both the last-mentioned forms have a comparatively simple pineal organ.

It will be noted that among the pineal organs of various teleosts listed above, only a very few showed an actual communication between the lumen of the pineal and that of the third ventricle. However, the species in which positive identification of a communication channel was made were scattered throughout the phylogenetic series. In the opinion of the present author, an open communication exists in all species of teleosts. The actual opening is extremely difficult to find, even in serial sections, and is so small in some species that it can be confined within a 7- $\mu$  section and thus remain invisible. The opening into the ventricle in *Strongylura* that can be seen in plate 2, figure 2, demonstrates the relative size of the channel compared with the cellular elements surrounding it. *Strongylura* is one of those species that exhibit a highly developed pineal organ. However, the communication was also found in *Hippocampus* which has one of the most rudi-

mentary organs in the series studied herein.

The communicating channel therefore permits the pineal secretion to enter the third ventricle where it becomes one of the components of the cerebrospinal fluid. The appearance of pineal secretion in neighboring blood vessels described by Friedrich-Freksa (1932) was not confirmed in the present study. Although this apocrine secretion is elaborated in the brain, of which the pineal is a part, it is not a true neurosecretion. Scharrer (1954) has defined neurosecretory cells as neurons with Nissl substance, dendrites, axons, and neurofibrils as well as granules and droplets of secretion. The pineal cells responsible for the apocrine secretion described do not conform to the description of neurons.

Although the teleost pineal has been established as a secretory gland, it is a question whether or not it produces a hormone. The obvious hormonal disturbances described by Pflugfelder (1953) as following pineal extirpation in *Lebistes* were not confirmed here in *Astyanax*. Implantation of *Atherina* pineals in *Cyprinodon* did not cause any changes in pigmentation in the hosts. Thus the effects shown for other classes of vertebrates and by Pflugfelder for *Lebistes* that indicate some relationship between the pituitary and the pineal were not obtained in the teleosts described herein.

Tilney and Warren (1919) have devoted most of their discussion to a consideration of the phylogenetic importance of both pineal and parietal bodies. Their conclusion is that

the epiphyseal anlage is "pluripotential in its derivatives," representing an inherent impulse towards the development of a glandular organ. The parietal anlage, on the other hand, exhibits a tendency towards the development of a parietal eye, or purely sensory organ. The teleost pineal obviously demonstrates both functions. Its sensory character is deduced from the behavior of fishes in response to varying light conditions and experimental procedures, and also the complex innervations suggest a sensory function. It is equally obvious that a secretory function is present.

In agreement with von Frisch (1911b) the present cytological studies did not reveal any cells with diagnostic features of sensory cells in the lining of the pineals of teleosts. The teleost pineal is a glandular organ the secretion of which is apocrine in nature. Except for the single report of Pflugfelder (1953) there is no evidence to show that the gland is endocrine in nature. Changes in pigmentation in amphibians and changes in endocrine glands of higher vertebrates attributed to administration of excess pineal material were brought about by the use of mammalian powders or extracts. The teleost pineal delivers glycogen and glycoprotein to the cerebrospinal fluid; both materials are useful for general metabolic purposes. Any endocrine function of the teleost pineal has still to be confirmed, and further experimental work should be confined to material of teleost origin, omitting the use of avian and mammalian derivatives which are obviously endocrine in nature.

## PART 2

Pickford and Atz (1957) have divided the teleosts into two groups according to their chromatophore reactions to pituitary preparations. Group I includes those fishes that always react to active pituitary preparations by the dispersion of pigment granules within the melanophores. Although unresponsive to purified intermedin, group II has again been divided into three categories: IIa is characterized by a great sensitivity to the melanophore-concentrating hormone and little or no response to intermedin; IIb is characterized by responses which are either melanophore

dispersing or concentrating; and IIc is characterized by dispersion of pigment granules in lipophores only. These authors consider that the fishes of group IIc also belong in group IIa, as general darkening would obscure the lipophore response. This is not necessarily true. A fish in which both xanthophores and melanophores are in a dispersed condition presents a much different color from that of one in which melanophores only are dispersed.

The lipophores and melanophores respond in the same manner, by dispersion of pig-



ment granules, to intermedin among the fishes of group I. Among the fishes of group II, lipophores are dispersed by intermedin, but melanophores are unresponsive. Erythrophore concentration was reported by Smith and Smith (1934) in *Scorpaena ustulata* after the injection of extracts of fresh fish hypophyses, and this seems to be the only report describing such a reaction in lipophores.

The guanophores of teleosts are of two varieties: the iridophore which contains refractive crystals and the leucophore which contains opaque white granules. There is some question as to whether there is actual movement of the crystals in the iridophore (Foster, 1933, 1937), but the opaque granules of the leucophore disperse or concentrate in response to background color and in most cases disperse under the influence of adrenalin. There is obviously some response in the iridophore to lighten the color of fishes equipped with these cells under the influence of adrenalin, as can be seen in *Strongylura* (pl. 2, fig. 3) and in *Apogonichthys* (pl. 10, fig. 1). Fries (1943) found that hypophysectomy had no effect on leucophore responses in *Fundulus*, and it is possible that these chromatophores are under nervous control exclusively. Further investigations of the physiology of the guanophores is necessary before conclusions can be drawn.

Recently it has been shown that pituitary hormones other than intermedin are involved in the pigmentary reactions of teleosts, although Thing (1953) suggests that this is due to contamination of various hypophysial preparations with intermedin. In the fresh-water minnow *Phoxinus*, Kohler (1952) was able to produce nuptial coloration with ACTH, affecting not only the melanophores but the xanthophores and erythrophores as well. Hewer (1926) obtained dispersion in xanthophores and erythrophores in *Phoxinus* after "infundin" injections. Dispersion of pigment in xanthophores and erythrophores has been noted by Meyer (1931) after injection of "pituitrin" and "hypophysin" in gobiids and pleuronectids.

Many fish species react to administered fish pituitary substance by concentration rather than dispersion of pigment granules in melanophores. This was first reported by Hogben (1942) who believed that some part

of the pituitary other than the pars intermedia produced a hormone "W substance" capable of aggregating pigment in melanophores or antagonizing the action of intermedin. Hogben thought that the visual and other reactions of fishes kept on white backgrounds may interfere with melanophore responses to injected pituitary substances, overriding their effects, and that such assays should be tried only on pale fishes kept in darkness. This seems to be true only to a certain extent. The intense response of the catfish to intermedin administration, for example, will take place regardless of visual cues; the pale, eyed fish in darkness is also responding to environmental cues which could override the influence of injected hormones. Weisel (1950) considers that the concentration of melanin pigment after fish pituitary administration may have a phylogenetic significance, as he found only the more highly specialized fishes to react in this way.

Probably none of the various reports in the literature is strictly comparable because of a lack of specificity in the hormones involved. Mammalian pituitary hormones have frequently been observed to give unpredictable results in fishes. ACTH, while it stimulates adrenal cortical hypertrophy in teleosts, is not nearly so potent in this regard as fresh carp pituitary (Rasquin, 1951), and the administration of mammalian gonadotropins is notoriously unreliable in bringing fishes to reproductive condition. In the present experiments with mammalian hormones of anterior lobe origin, the marine fishes appear to have responded not to individual hormones but to the group as a whole. Why the fresh-water species respond specifically to MSH with a reaction of melanophores and not to the whole group of pituitary fractions with xanthophore reaction as marine fishes do is a question. Obviously some fresh-water species respond to intermedin with xanthophore and erythrophore dispersion. In Europe, *Phoxinus* has been used as an assay animal for intermedin, and the reaction is based on the erythrophore response and not the melanophore response.

Sulman and Eviator (1956) have shown that there are two, and possibly three, chromatophore hormones in the dog pituitary

which cause melanophore dispersion in amphibians. They were able to suppress the activity of the melanophore hormone of the anterior lobe by suppressing the output of ACTH after injections of cortical hormones. They conclude that this hormone is elaborated by the same basophile cells that elaborate corticotrophin and associate the pigmentation of human patients with Addison's disease with excess production of the chromatophore hormone of the anterior lobe.

In the case of the stimulation of "nuptial colors" (Hewer, 1926; Burrows, Palmer, and Newman, 1952) it is probable that all the chromatophores are involved. Pituitary extracts may not be stimulating chromatophores directly, but may be stimulating sex-hormone production which is responsible for the change in coloration. The effect on chromatophores would, therefore, be only secondary.

It is well known that xanthophores and melanophores are not affected in the same way by various humoral agents (Matthews,

1933; Abramowitz, 1936; Fries, 1943). Adrenalin may concentrate the granules within melanophores and disperse them in xanthophores at the same time (Abolin, 1925). It is not believed in the present instance that any adrenalin-like substance was released as a result of the mammalian hormone injections, because the melanophores did not respond to the injections. Obviously, some marine fishes do not respond to adrenalin administration by pigmentary reactions, but *Bathygobius* does not belong to these groups; immediate concentration of melanophores follows adrenalin injection in this goby (pl. 10, fig. 2).

The present report indicates a certain specificity of the pituitary hormones, showing that different responses to mammalian hormones can be evoked even among relatively closely related teleosts. It shows again that experimental results of endocrinological work done on one species of fish should not be assumed to apply to the entire class.

## SUMMARIES

### PART 1

1. THE HISTOMORPHOLOGY of the pineal area is described for 33 species of teleosts. The pineal organ is composed of an end-vesicle, a stalk, and a short pedicle, but variations in size and development of the vesicle and stalk create great differences in the morphology of the organ in different species.

2. In general, those species in which the pineal organ is exposed to light passing through transparent overlying tissues, or in which the pineal area can be exposed to light by concentration of pigment granules in overlying chromatophores, show a specialization of the pineal organ. The end-vesicle in particular covers a wide area, spreading over parts of the forebrain and optic lobes, with the surface area of pineal cells greatly increased by invaginations and convolutions of the walls. Those species in which the pineal organ is deeply buried under many tissue layers are found to have a more simplified structure, with the vesicle limited to a simple sac.

3. Cytological study shows that the pineal is a complex organ probably having both sensory and secretory functions. Nerve endings seen near the pineal cells are connected with ganglion cells. Fibers from the ganglion cells progress down the walls of the stalk and enter the habenular commissure. Two types of epithelioid cells form the inner walls of the pineal vesicle and stalk, resting on a connective-tissue, basement membrane. These epi-

thelioid or pineal cells either are sloughed off into the lumen, or parts of them are broken off into the lumen to form an apocrine secretion.

4. The apocrine secretion of the pineal can be delivered to the cerebrospinal fluid through the opening in the base of the stalk or pedicle that connects the lumen of the pineal stalk with the third ventricle of the diencephalon.

5. The pineal secretion in teleosts is composed, at least in part, of glycogen and glycoprotein. In some species, as *Atherina stipes*, the pineal cells accumulate glycogen during hours of darkness. The quantity of glycogen decreases during hours of light.

6. Intraperitoneal implantation of fresh *Atherina* pineals caused no observable effect on pigmentation in *Cyprinodon*.

7. Pinealectomy in *Astyanax* had no effect on pituitary, thyroid, or gonads and no observable effect on pigmentation. No significant changes in phototaxis were observed, owing perhaps to the dense pigmentation over the top of the head in this species.

8. Although no specific sensory cells were discovered in the teleost pineals, the behavior of fishes shows a sensory influence of this area of the brain. Other results of this report indicate that there is no evidence for an endocrine function of the secretion of the teleost pineal gland.

### PART 2

1. Thirty-five species of teleosts were injected with adrenalin or with intermedin or with both hormones at different times.

2. Fishes can be grouped in three categories according to the reaction of the melanophore system to adrenalin: (1) fishes in which adrenalin causes concentration of pigment granules within all the melanophores, (2) fishes in which only the internal melanophores show pigment concentration, and (3) fishes in which none of the melanophores is affected by adrenalin injection.

3. In some species, dermal melanophores are not concentrated by the effects of adrenalin injection but are responsive to the direct application of the hormone to the skin.

4. The lipophores respond to adrenalin either by dispersion or concentration of pigment granules, depending upon the species.

5. Leucophores respond to adrenalin injection by dispersion of guanin granules.

6. Dispersion of melanin granules in chromatophores as a response to intermedin was confined to *Ameiurus*, *Gambusia*, *Asty-*

*anax*, and *Atherina* among the species used for this report. All other species showed no reaction of melanophores to intermedin.

7. All species responded to intermedin by dispersion of lipophores.

8. Six mammalian anterior pituitary preparations were tested for their effects on the pigmentation of the fresh-water characin *Astyanax mexicanus* and the marine goby *Bathygobius soporator*. These hormones were FSH, LH, TSH, MSH, prolactin, and growth hormone. In addition, ACTH was used on *Bathygobius*.

9. MSH administration caused dispersion of melanin granules in the melanophores of *Astyanax*. No pigmentary reactions were

noted in this species after injection of any other pituitary fractions.

10. Administration of all the anterior lobe preparations caused dispersion of pigment granules in the xanthophores of *Bathygobius*. No reaction was noted in the melanophores.

11. MSH was administered to eight other species with the following results: in fresh water, *Ameiurus nebulosus*, dispersion of melanophores; in sea water, *Gambusia* sp. and *Atherina stipes*, dispersion of melanophores; *Cyprinodon baconi* and *Monacanthus ciliatus*, no reaction; *Gambusia* sp., *Lutianus apodus*, *Irideo bivitatta*, and *Chaetodon striatus*, dispersion of xanthophores or erythrophores.

## BIBLIOGRAPHY

- ABOLIN, L.  
1925. Beeinflussung des Fischfarbwechsels durch Chemikalien. I. Infundin- und Adrenalinwirkung auf die Melano- und Xanthophoren der Elritze (*Phoxinus laevis* Ag.). Arch. Mikros. Anat., vol. 104, pp. 667-697.
- ABRAMOWITZ, A. A.  
1936. The non-identity of the neurohumors for the melanophores and the xanthophores of *Fundulus*. Amer. Nat., vol. 70, pp. 372-378.
- BREder, C. M., JR., AND P. RASQUIN  
1947. Comparative studies in the light sensitivity of blind characins from a series of Mexican caves. Bull. Amer. Mus. Nat. Hist., vol. 89, pp. 325-251.  
1950. A preliminary report on the role of the pineal organ in the control of pigment cells and light reactions in recent teleosts. Science, vol. 111, pp. 10-12.  
1955. Further notes on the pigmentary behavior of *Chaetodipterus* in reference to background and water transparency. Zoologica, New York, vol. 40, pp. 85-90.
- BURROWS, R. E., D. D. PALMER, AND H. W. NEWMAN  
1952. Effects of injected pituitary material upon the spawning of blueback salmon. Progr. Fish-Culturist, vol. 14, pp. 113-116.
- CHAVIN, W.  
1956. Pituitary-adrenal control of melanization in xanthic goldfish, *Carassius auratus*, L. Jour. Exp. Zool., vol. 133, pp. 1-45.
- DEAN, B.  
1891. The pineal fontanelle of placoderm and catfish. 19th Rept. Fish Comm. New York, pp. 309-333.
- FISCHER, O.  
1943. Preparation of an active fraction of the pineal gland. Endocrinology, vol. 33, pp. 116-117.
- FOSTER, K. W.  
1933. Color changes in *Fundulus* with special reference to the color changes of the iridosomes. Proc. Natl. Acad. Sci., vol. 19, pp. 535-340.  
1937. The blue phase in the color changes of fish with special reference to the role of the guanin deposits in the skin of *Fundulus heteroclitus*. Jour. Exp. Zool., vol. 77, pp. 169-213.
- FRIEDRICH-FREKSA, H.  
1932. Entwicklung, Bau und Bedeutung der parietalgegend bei Teleosteen. Zeitschr. Wiss. Zool., vol. 141, pp. 52-142.
- FRIES, E. F. B.  
1942. White pigmentary effectors (leucophores) in killifishes. Proc. Natl. Acad. Sci., vol. 28, pp. 396-401.  
1943. Pituitary and nervous control of pigmentary effectors, especially xanthophores, in killifish (*Fundulus*). Physiol. Zool., vol. 16, pp. 199-212.
- FRISCH, K. VON  
1911a. Beiträge zur Physiologie der Pigmentzellen in der Fischhaut. Arch. Ges. Physiol., vol. 138, pp. 319-387.  
1911b. Das Parietalorgan der Fische als funktionierendes Organ. Sitzber. Gesell. Morph. Physiol., vol. 27, pp. 16-18.
- GREGORY, W. K.  
1933. Fish skulls: a study of the evolution of natural mechanisms. Trans. Amer. Phil. Soc., new ser., vol. 23, pp. 75-481.
- GREGORY, W. K., AND G. M. CONRAD  
1938. The phylogeny of the characin fishes. Zoologica, New York, vol. 23, pp. 319-360.
- GRUNEWALD-LOWENSTEIN, M.  
1952. Studies on the function of the pineal body. Exp. Med. and Surg., vol. 10, pp. 135-154.  
1956. Influence of light and darkness on the pineal body in *Astyanax mexicanus* (Filippi). Zoologica, New York, vol. 41, pp. 119-128.
- HANDRICK, K.  
1901. Zur Kenntnis des Nervensystems und der Leuchtorgane von *Argyropelecus hemigymnus*. Zoologica, Stuttgart, vol. 13, pp. 1-69.
- HEWER, H. R.  
1926. Studies in colour changes of fish. 1. The action of certain endocrine secretions in the minnow. Brit. Jour. Exp. Biol., vol. 3, pp. 123-140.
- HILL, C.  
1894. The epiphysis of teleosts and *Amia*. Jour. Morph., vol. 9, pp. 237-268.
- HOAR, W. S.  
1955. Phototactic and pigmentary responses of sockeye salmon smolts following injury to the pineal organ. Jour. Fish. Res. Board of Canada, vol. 12, pp. 178-185.

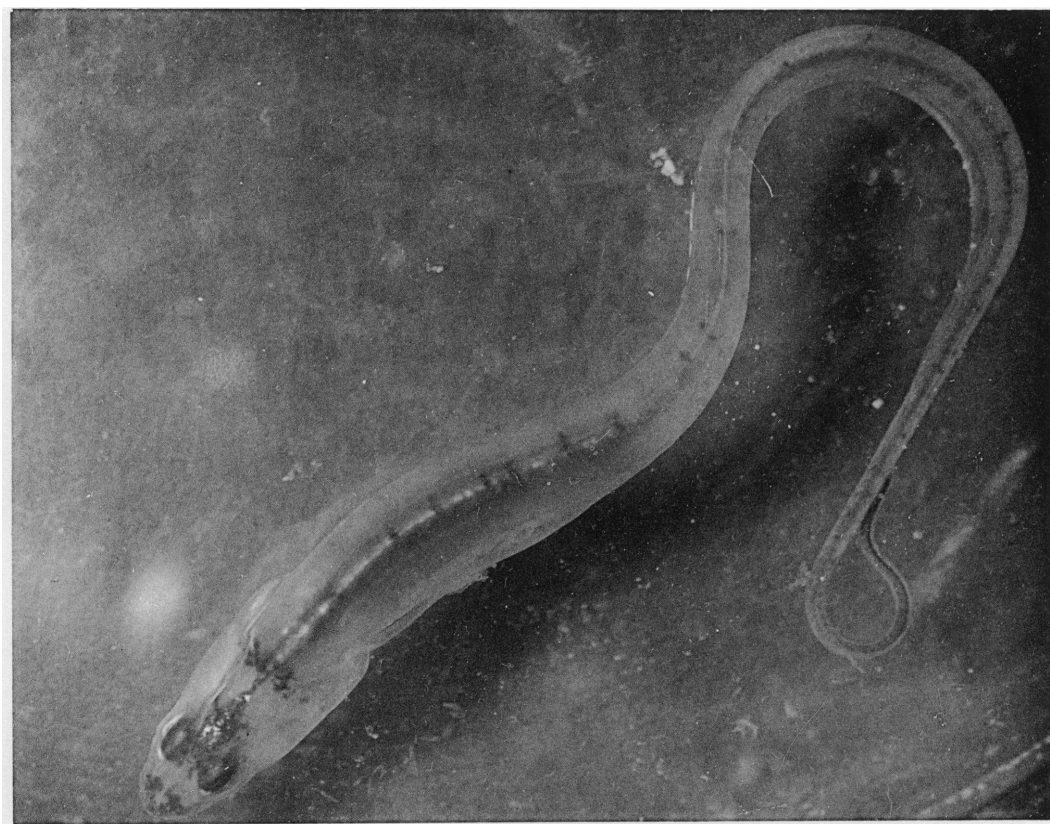
- HOGBEN, L.  
1942. Chromatic behaviour. Proc. Roy. Soc. London, ser. B, vol. 131, pp. 111-136.
- HOLMGREN, N.  
1918a. Zum Bau der Epiphyse von *Squalus acanthias*. Arkiv Zool., vol. 11, no. 23, pp. 1-28.  
1918b. Über die Epiphysennerven von *Clupea sprattus* und *harengus*. Ibid., vol. 11, no. 25, pp. 1-5.  
1920. Zur Anatomie und Histologie des Vorder- und Zwischenhirns der Knochenfische. Acta Zool., vol. 1, pp. 137-315.
- KAPPERS, J. A.  
1957. On the problem of the presence of a paraphysis cerebri in selachians. Pubbl. Staz. Zool. Napoli, vol. 29, pp. 41-70.
- KOHLER, V.  
1952. Die Wirkung des Adrenocorticotropins auf die Lipophoren der Pfrille (*Phoxinus laevis*). Die Naturwissenschaften, vol. 39, pp. 554.
- LEDUC, E. H., AND G. B. WISLOCKI  
1952. The histochemical localization of acid and alkaline phosphatases, non-specific esterase and succinic dehydrogenase in the structure comprising the hematoencephalic barrier of the rat. Jour. Comp. Neurol., vol. 97, pp. 241-279.
- MCCORD, C. P., AND F. P. ALLEN  
1917. Evidences associating pineal gland function with alterations in pigmentation. Jour. Exp. Zool., vol. 23, pp. 207-224.
- MATTHEWS, S. A.  
1933. Color changes in *Fundulus* after hypophysectomy. Biol. Bull., vol. 64, pp. 315-320.
- MEYER, E.  
1931. Versuche über den Farbwechsel von *Gobius* und *Pleuronectes*. Zool. Jahrb., Abt. Zool. Physiol., vol. 49, pp. 231-270.
- MOSZOWSKA, A.  
1947. Difference of activity in pineal glands of some mammals and fowls. Ann. Endocrinol., vol. 8, pp. 138-140.
- PARKER, G. H.  
1948. Animal colour changes and their neurohumours. Cambridge, Cambridge University Press, 377 pp.
- PFLUGFELDER, O.  
1953. Wirkungen der Epiphysectomie auf die Postembryonalentwicklung von *Lebistes reticulatus* Peters. Arch. Entwickl. Mech., vol. 146, pp. 115-136.  
1954. Wirkungen partieller Zerstörungen der Parietalregion von *Lebistes reticulatus*. Ibid., vol. 147, pp. 42-60.
- PICKFORD, G. E., AND J. W. ATZ  
1957. The physiology of the pituitary gland of fishes. New York, New York Zoological Society.
- RASQUIN, P.  
1951. Effects of carp pituitary and mammalian ACTH on the endocrine and lymphoid systems of the teleost *Astyanax mexicanus*. Jour. Exp. Zool., vol. 117, pp. 317-357.
- RIVAS, L. R.  
1953. The pineal apparatus of tunas and related scombrid fishes as a possible light receptor controlling phototactic movements. Bull. Marine Sci. Gulf and Caribbean, vol. 3, pp. 168-180.
- SAGEMEHL, M.  
1885. Beiträge zur vergleichenden Anatomie der Fische. III. Das Cranium der Characinen nebst allgemeinen Bemerkungen über die mit einem Weber'schen Apparat versehenen Physostomenfamilien. Morph. Jahrb., vol. 10, pp. 1-119.
- SCHARRER, E.  
1928. Die Lichtempfindlichkeit blinder Elritzen. (Untersuchungen über das Zwischenhirn der Fische. I.) Zeitschr. Vergl. Physiol., vol. 7, pp. 1-38.  
1954. Neurosecretion in the vertebrates: a survey. Pubbl. Staz. Zool. Napoli, vol. 24, suppl., pp. 8-10.
- SCHÖNHERR, J.  
1955. Über die Abhängigkeit der Instinkt-handlungen von Vorderhirn und Zwischenhirn (Epiphyse) bei *Gasterosteus aculeatus* L. Zool. Jahrb. vol. 65, pp. 357-386.
- SHANES, M., AND R. F. NIGRELLI  
1941. The chromatophores of *Fundulus heteroclitus* in polarized light. Zoologica, New York, vol. 26, pp. 237-240.
- SHELLABARGER, C. J.  
1953. Observations of the pineal in the white Leghorn capon and cockerel. Endocrinology, vol. 51, pp. 152-154.
- SIMMONET, H., AND J. STERNBERG  
1951. Le rôle endocrinien de l'épiphyse. I. L'épiphyse et le métabolisme du phosphore. Rev. Canadienne Biol., vol. 9, pp. 407-421.
- SIMMONET, H., AND L. THIÉBLOT  
1951. Recherches expérimentales sur la physiologie de la glande pineale. Acta Endocrinol., vol. 7, pp. 306-320.
- SIMMONET, H., L. THIÉBLOT, AND T. MELIK  
1951. Influence de l'épiphysé sur l'ovaire de la jeune rat. Ann. Endocrinol., vol. 12, pp. 202-205.

- SIMMONET, H., L. THIÉBLOT, T. MELIK, AND V. SEGAL  
1953. Influence de l'épiphyséctomie sur la structure de l'hypophyse. *Bull. Assoc. Anat.*, no. 72, pp. 126-129.
- SIMMONET, H., L. THIÉBLOT, AND V. SEGAL  
1952. Interrelation épiphysio-hypophysaire et effect expansomelanophorique. *Ann. Endocrinol.*, vol. 13, pp. 340-344.
- SMITH, D. C., AND M. T. SMITH  
1934. Observations on the erythrophores of *Scorpaena ustulata*. *Biol. Bull.*, vol. 34, pp. 45-58.
- SOKOL, H. W.  
1953. Selective staining of the pituitary gland of teleosts demonstrating at least six tinctorial cell types in the adenohypophysis. *Anat. Rec.*, vol. 117, p. 582. (Also personal communication.)
- STEARNER, S. P.  
1946. Pigmentation studies in salamanders, with especial reference to the changes at metamorphosis. *Physiol. Zool.*, vol. 19, pp. 375-404.
- STERZI, G.  
1902. Recherches sur l'anatomie comparée et sur l'ontogenèse des méninges. *Arch. Italiano Biol.*, vol. 37, pp. 257-269.
- STUDNIČKA, F. K.  
1896. Beiträge zue Anatomie und Entwicklungsgeschichte des Vorderhirns der Cranioten. *Sitzungsber. Gesell. Wiss.*, no. 15, pp. 1-32.  
1905. Die Parietalorgane. In Oppel, Carl Albert (ed.), *Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbeltiere*. Jena, vol. 5, viii+254 pp.
- SULMAN, F. G., AND A. EVIATOR  
1956. The existence of two chromatophore hormones in the pituitary gland. *Acta Endocrinol.*, vol. 23, pp. 120-130.
- TERRY, R. J.  
1910. The morphology of the pineal region in teleosts. *Jour. Morph.*, vol. 21, pp. 321-358.
- THIÉBLOT, L.  
1954. Le rôle fonctionnel de l'épiphysé. *Rev. Canadienne Biol.*, vol. 13, pp. 189-207.
- THING, E.  
1953. Melanophore reaction and adrenocorticotrophic hormone. *Acta Endocrinol.*, vol. 13, pp. 29-38.
- TILNEY, F., AND L. F. WARREN  
1919. The morphology and evolutionary significance of the pineal body. *Mem. Amer. Anat.*, vol. 9, pp. 5-257.
- TRETJAKOFF, D.  
1915. Die Parietalorgane von *Petromyzon fluviatilis*. *Zeitschr. Wiss. Zool.*, vol. 113, pp. 1-112.
- TWITTY, V. C., AND D. BODENSTEIN  
1939. Correlated genetic and embryological experiments on *Triturus*. III. Further transplantation experiments on pigment development. IV. The study of pigment cell behavior in vitro. *Jour. Exp. Zool.*, vol. 81, pp. 357-398.
- VAN DE KRAMER, J. C.  
1952. On the function of the pineal organ. *Arch. Néerlandaises Zool.*, vol. 9, pp. 565-566.
- WEISEL, G. F.  
1950. The comparative effects of beef and teleost pituitary on chromatophores of cold-blooded vertebrates. *Biol. Bull.*, vol. 99, pp. 487-496.
- WISLOCKI, G. B., AND E. W. DEMPSEY  
1948. The chemical histology and cytology of the pineal and neurohypophysis. *Endocrinology*, vol. 42, pp. 56-72.

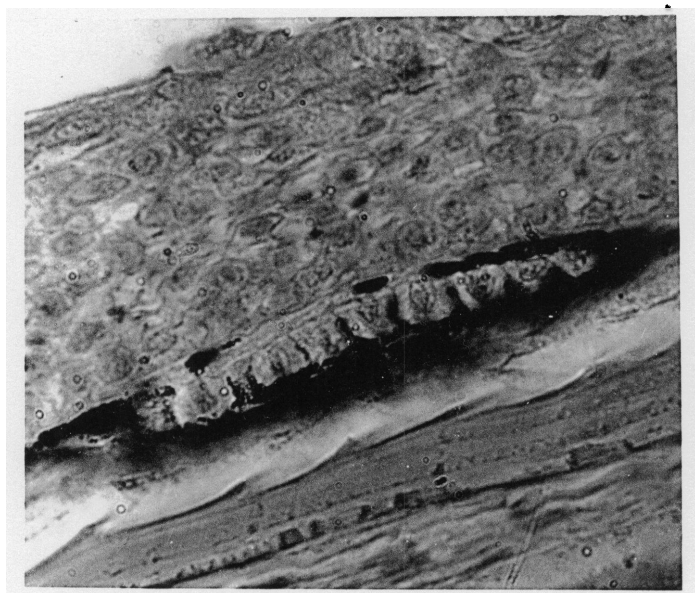


**PLATES 1-10**

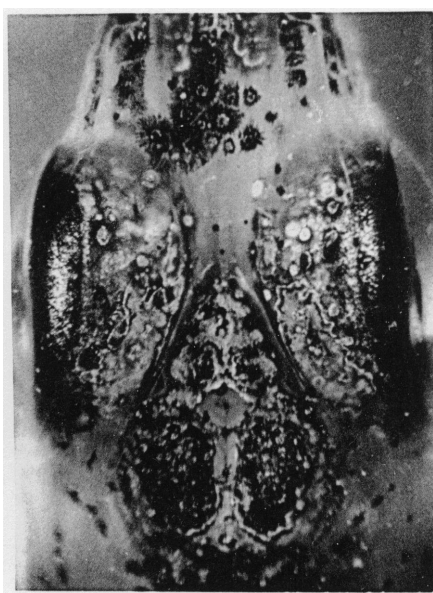




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1. Dorsal view of *Carapus bermudensis*, showing pattern formed chiefly by melanophores covering brain and spinal cord

2. Section through skin of *Tylosurus raphidoma*, showing a melaniridosome, and dendritic processes of the melanophore pushing up between cuboidal iridophores.  $\times 800$

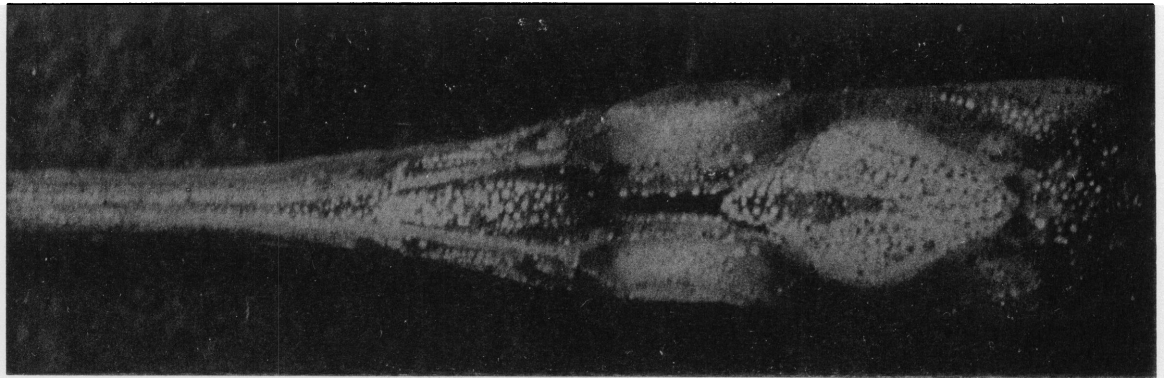
3. Dorsal view of head of *Jenkinsia lamprotaenia*, showing window-like, transparent area over pineal region



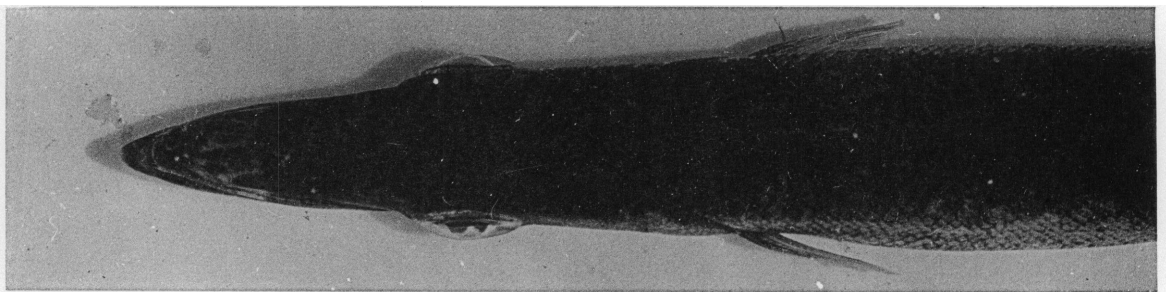
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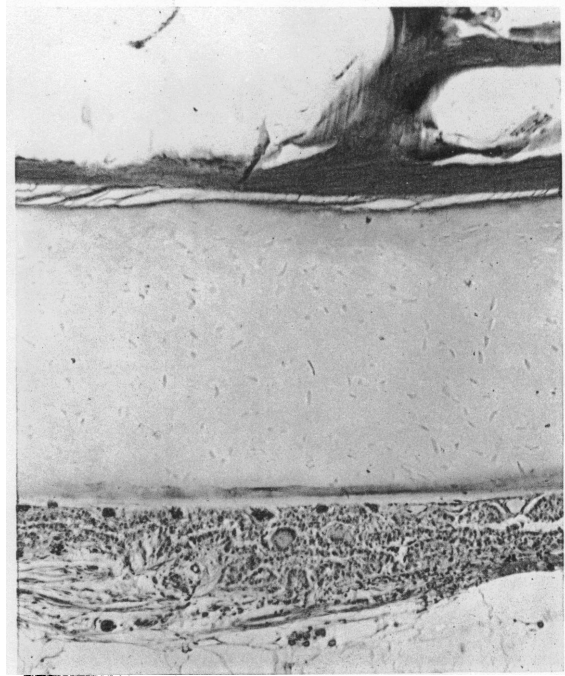


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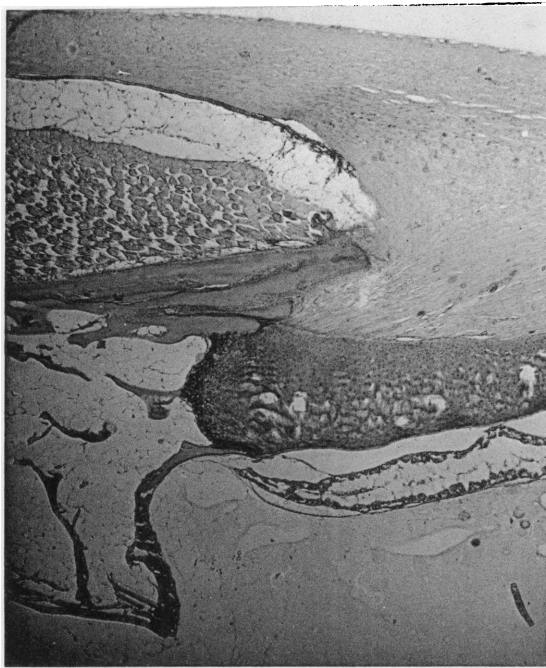
1. Transverse section through pineal organ of *Atherina stipes*, showing vesicle and stalk with its connection to habenular commissure.  $\times 85$
2. Longitudinal section through base of pineal organ of *Strongylura notata*, showing continuity of stalk lumen with that of third ventricle.  $\times 450$
3. Dorsal view of head of *Strongylura notata* after administration of adrenalin, showing how window-like area over pineal is opened for admission of light by concentration of granules in melanophores
4. Dorsal view of head of *Sphyræna barracuda* under urethane anesthesia, showing how dispersion of melanophores can form complete protective covering



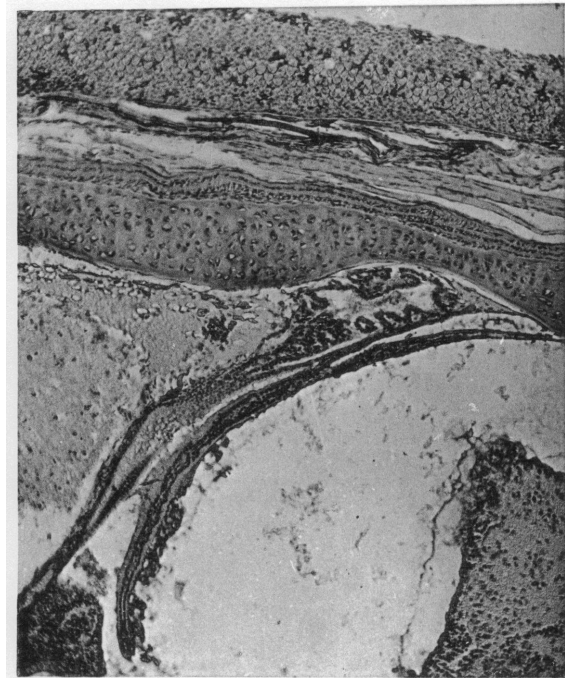
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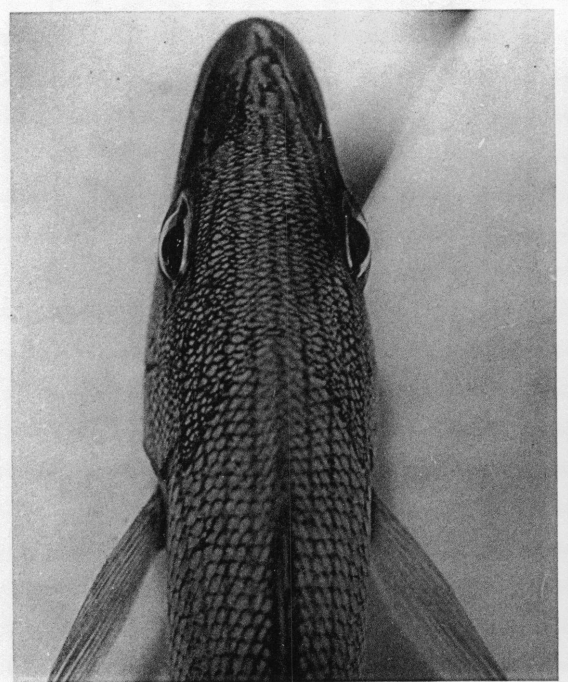
1. Sagittal section through pineal region of *Sphyræna barracuda*, showing wide lumen and close association of stalk with posterior wall of dorsal sac.  $\times 30$

2. Pineal vesicle of *Gerres cinereus*, showing highly convoluted walls, narrow lumen, and blood vessels in capsule.  $\times 85$

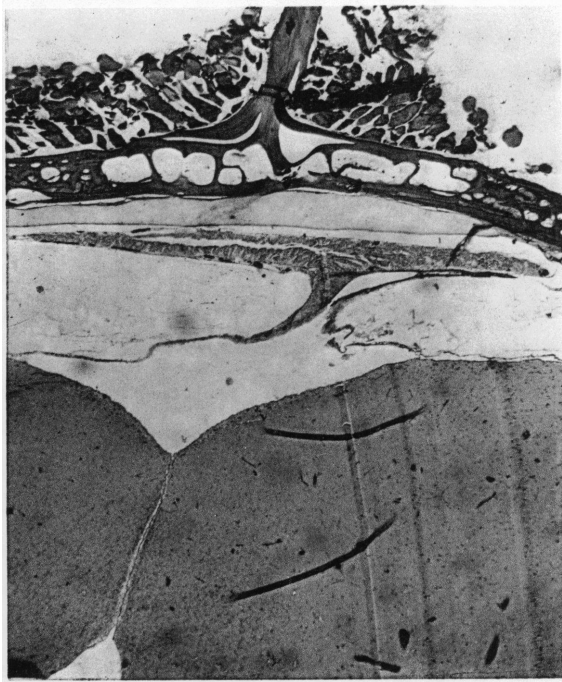
3. Pineal area of *Gymnosardus*, showing transition between tissues overlying pineal and those overlying rest of brain.  $\times 30$

4. Sagittal section of pineal region of *Nomeus gronovii*, showing modification of overlying tissues to be confined to a thinning of skull.  $\times 85$

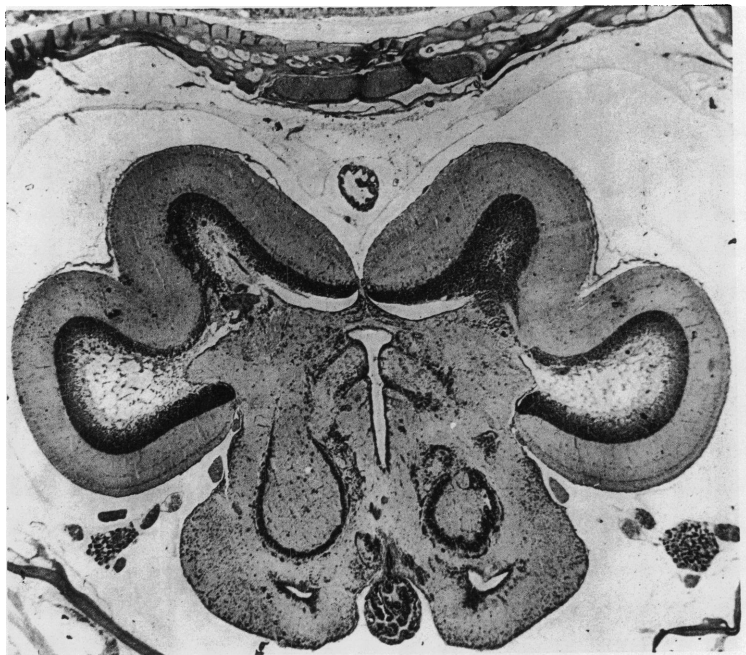




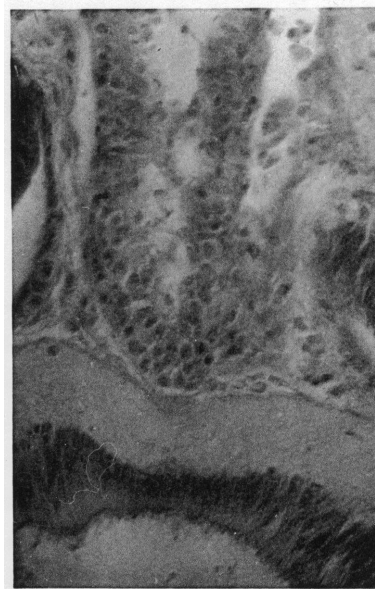
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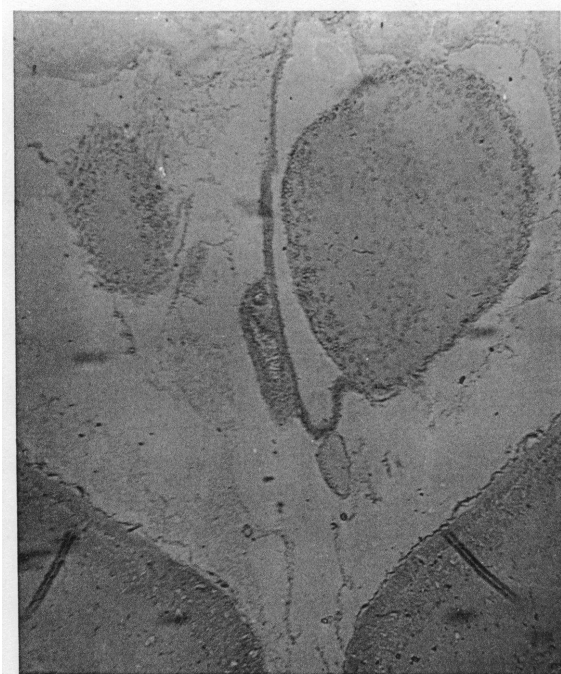
1. Dorsal view of head of *Haemulon sciurus*, showing lack of any modification of exterior over pineal region
2. Oblique transverse section through pineal vesicle of *Haemulon sciurus*, showing small size of organ.  $\times 30$
3. Transverse section through optic lobes of *Spheroides spangleri*. The pineal organ situated between optic lobes.  $\times 30$
4. Section through pineal stalk of *Spheroides* near base, showing cellular debris in lumen.  $\times 450$



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1. Transverse section of pineal region of immature *Histro*, showing how organ is situated well below dorsal limit of optic lobes

2. Sagittal section through pineal area of *Hippocampus reidi*, showing simple structure of organ in this species, and melanophores in pineal capsule

3. Transverse section through pineal area of *Monacanthus ciliatus*, showing organ buried well below dorsal level of frontal lobes

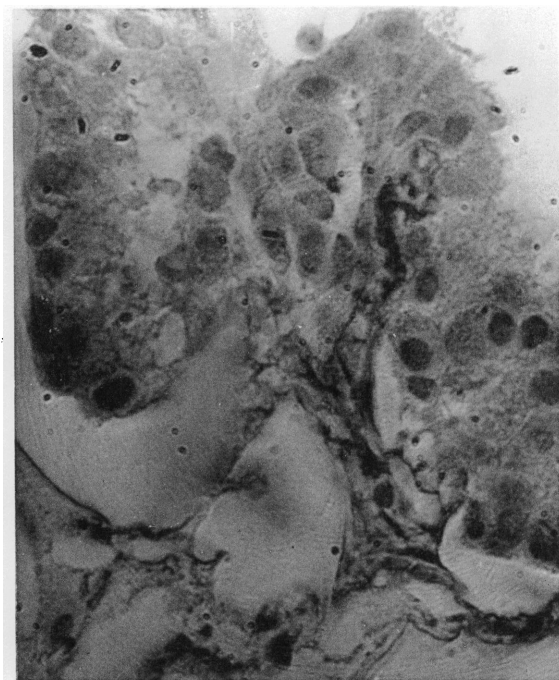
4. Transverse section through brain of *Monacanthus ciliatus*, showing habenular ganglia and small size of dorsal sac

All  $\times 85$

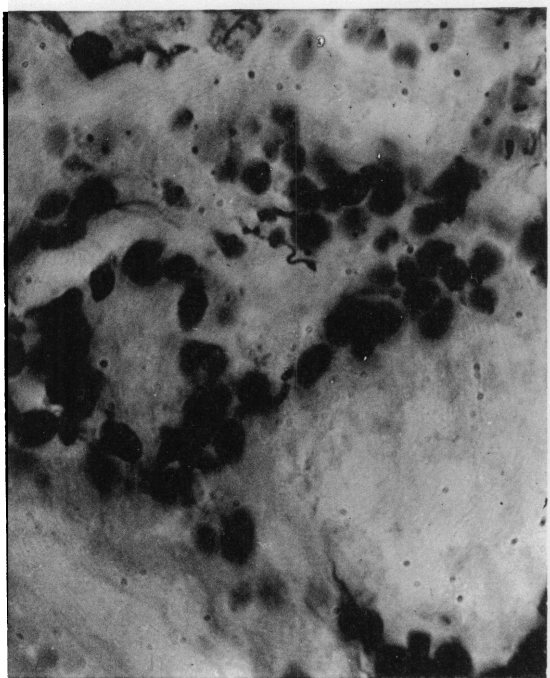




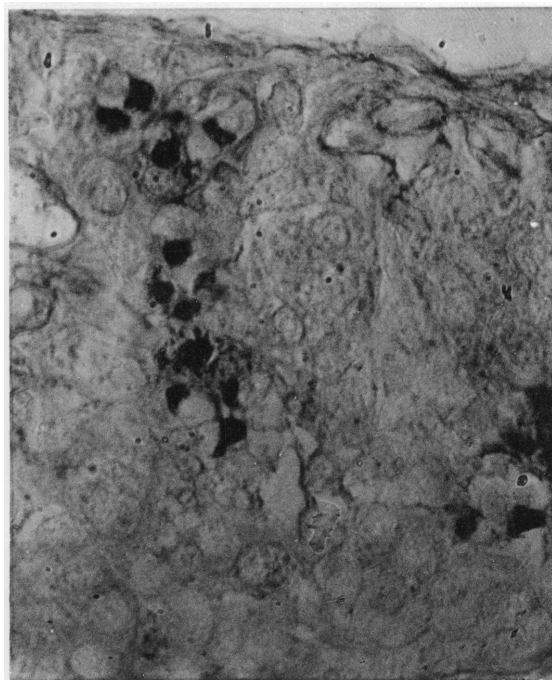
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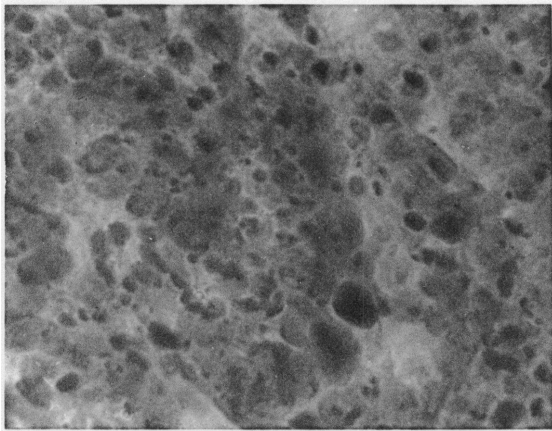
1. Section of pineal of *Astyanax mexicanus*, fixed in Bouin and stained with Masson's trichrome method, showing types of cells and so-called cell processes extending into lumen

2. Section through pineal of *Astyanax mexicanus* fixed in Maximow's fluid and stained with Heidenhain's azocarmine method, showing marked differentiation of basement membrane

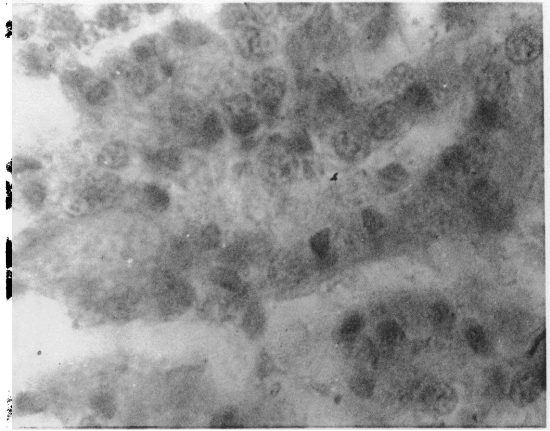
3. Section through pineal of *Atherina stipes*, stained with Bielschowsky's method, showing a free nerve ending

4. Section through pineal of *Cyprinodon baconii* stained with Sokol's modification of Halmi's stain, showing positive reaction or cellular debris in lumen

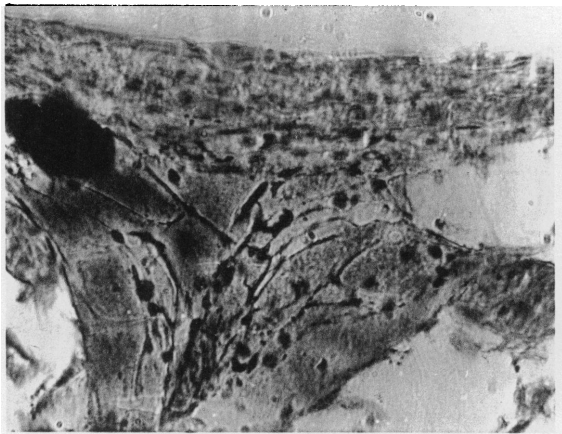
All  $\times 900$



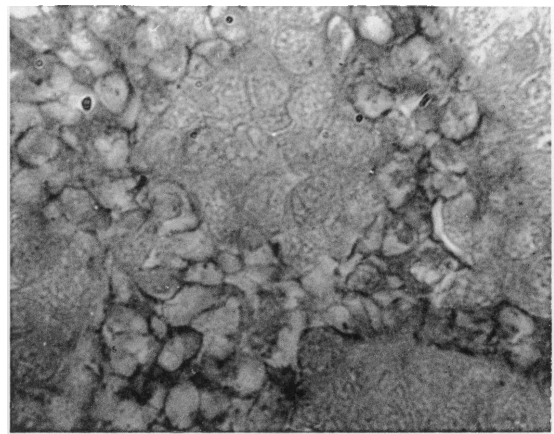
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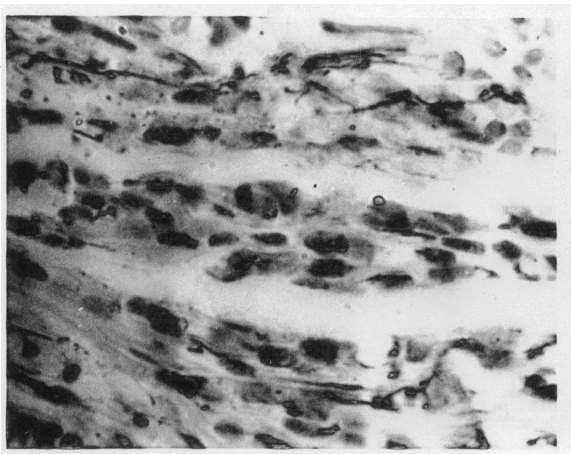
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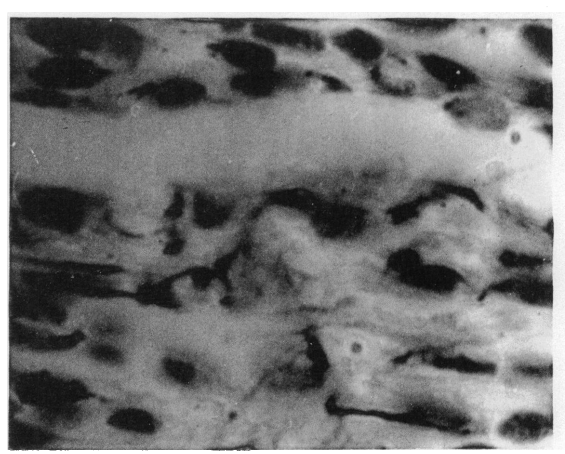
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1. Section through *Astyanax* pineal stained by the periodic acid-Schiff method, showing glycogen within cells and lumen. X 450

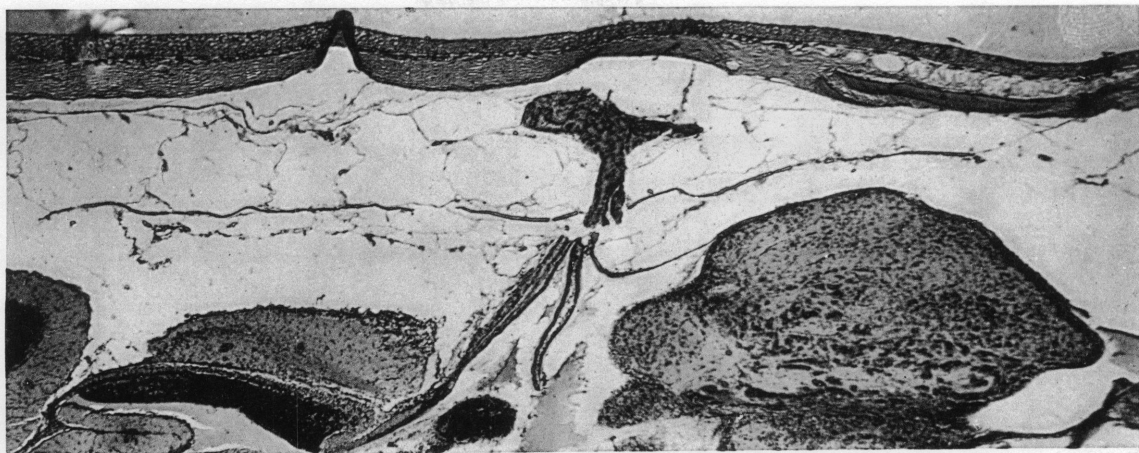
2. Section through *Astyanax* pineal stained similarly with PAS after saliva digestion. Absence of stained material indicates positively stained material of 1 to be glycogen. X 450

3. Section through base of vesicle of *Atherina stipes* stained by Bodian's silver-protargol method, showing ganglion cells. X 450

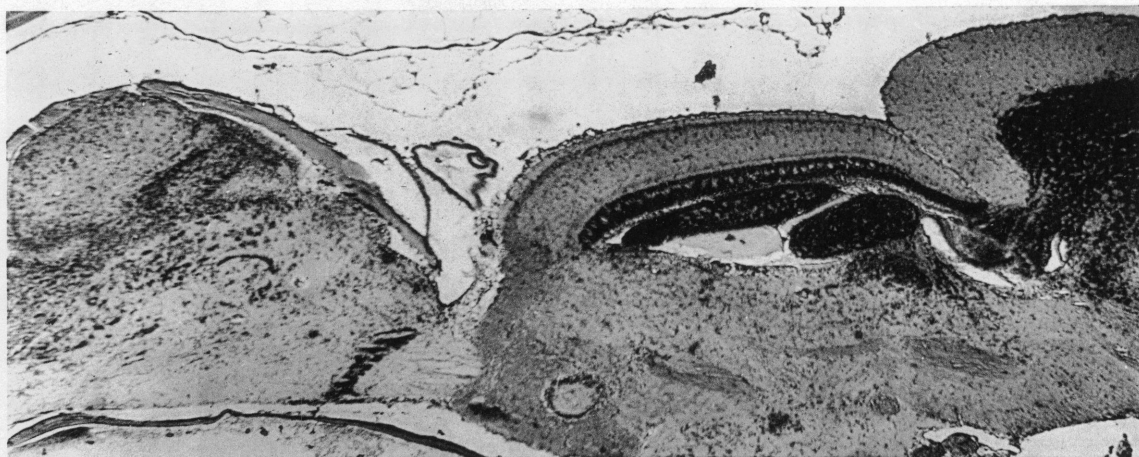
4. Section through *Astyanax* pineal stained with Gomori's aldehyde-fuchsin without previous oxidation, showing positive reaction in lumen. X 550

5. Section through *Atherina* pineal stained with Bodian's silver-protargol method, showing fine nerve fibers in walls. X 450

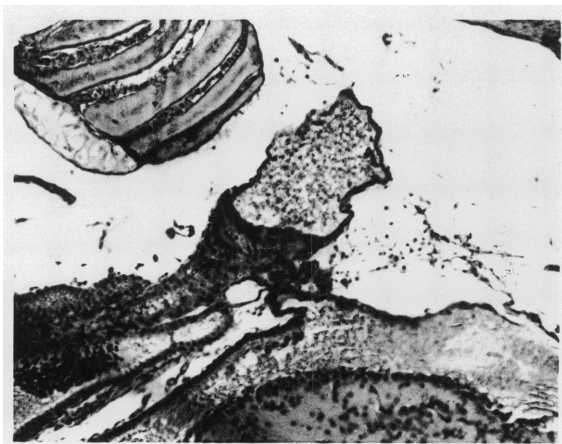
6. Detail of 5, showing nerve endings in walls of pineal. X 900



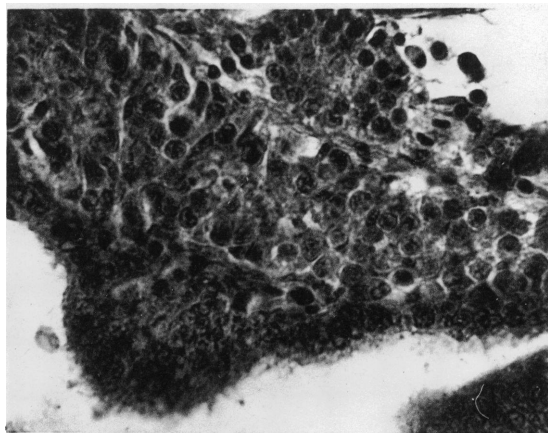
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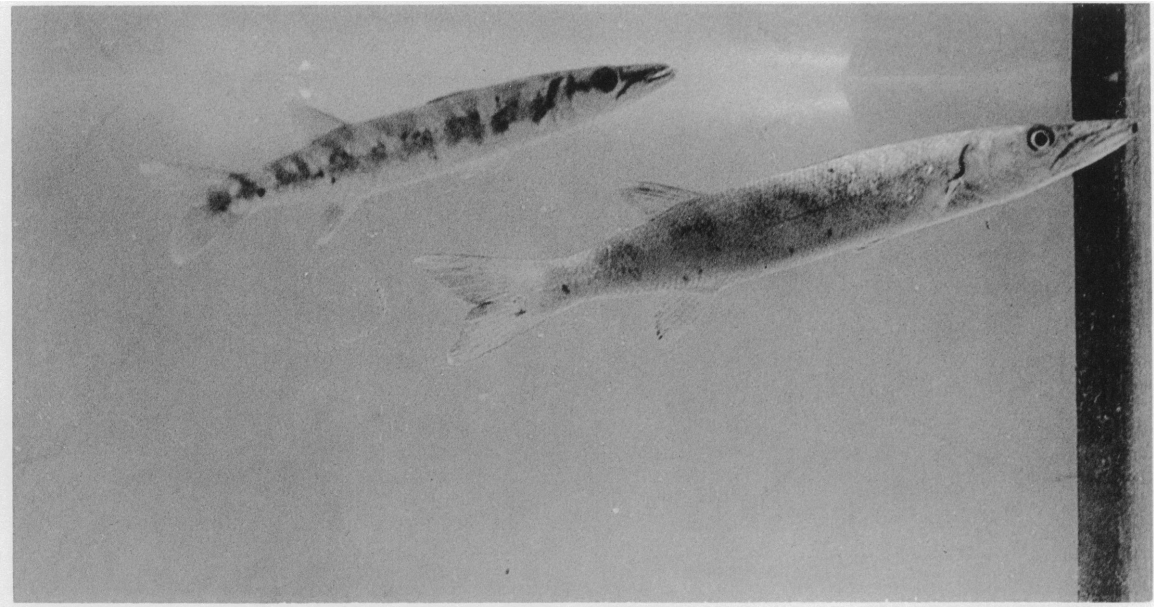


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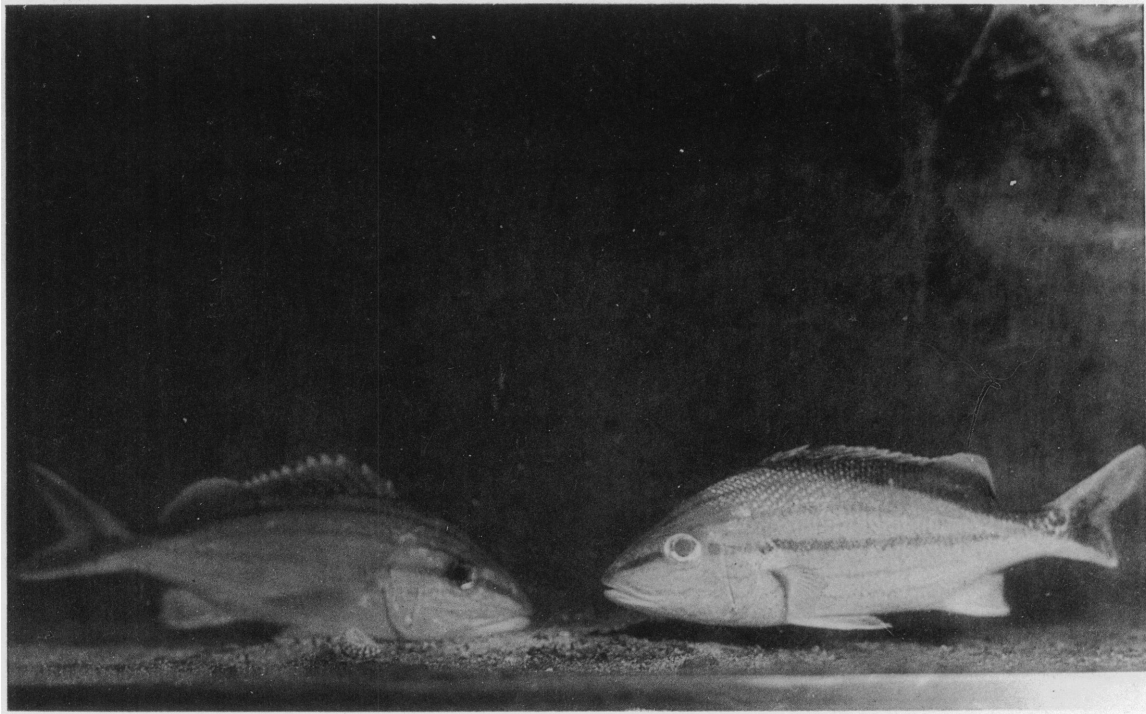


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1. Sagittal section through pineal region of brain of fish no. 6, showing intact organ. Section through stalk is an artifact.  $\times 30$
2. Sagittal section through pineal region of brain of fish no. 3, showing complete absence of organ. Small band of tissue in pineal region is part of dorsal sac, roof of third ventricle.  $\times 30$
3. Section showing incomplete removal of pineal of fish no. 9 with a large cyst in remains of vesicle.  $\times 140$
4. Section showing part of stalk remaining in fish no. 8 with absence of lumen and small size of cells.  $\times 600$



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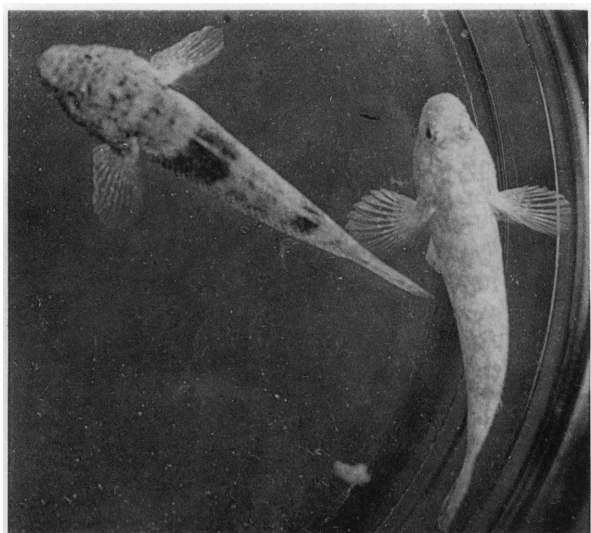
1. Two barracuda (*Sphyraena barracuda*), the larger of which was injected with adrenalin, showing white iris caused by concentration of melanophores compared with dark iris of non-injected control. The lighter color phase of injected fish is not a result of adrenalin. See text for full explanation

2. Two grunts (*Haemulon melanurum*). The fish on right shows white iris which is result of adrenalin injection, compared with dark iris of non-injected fish on left. Dermal melanophores were unaffected by adrenalin administration

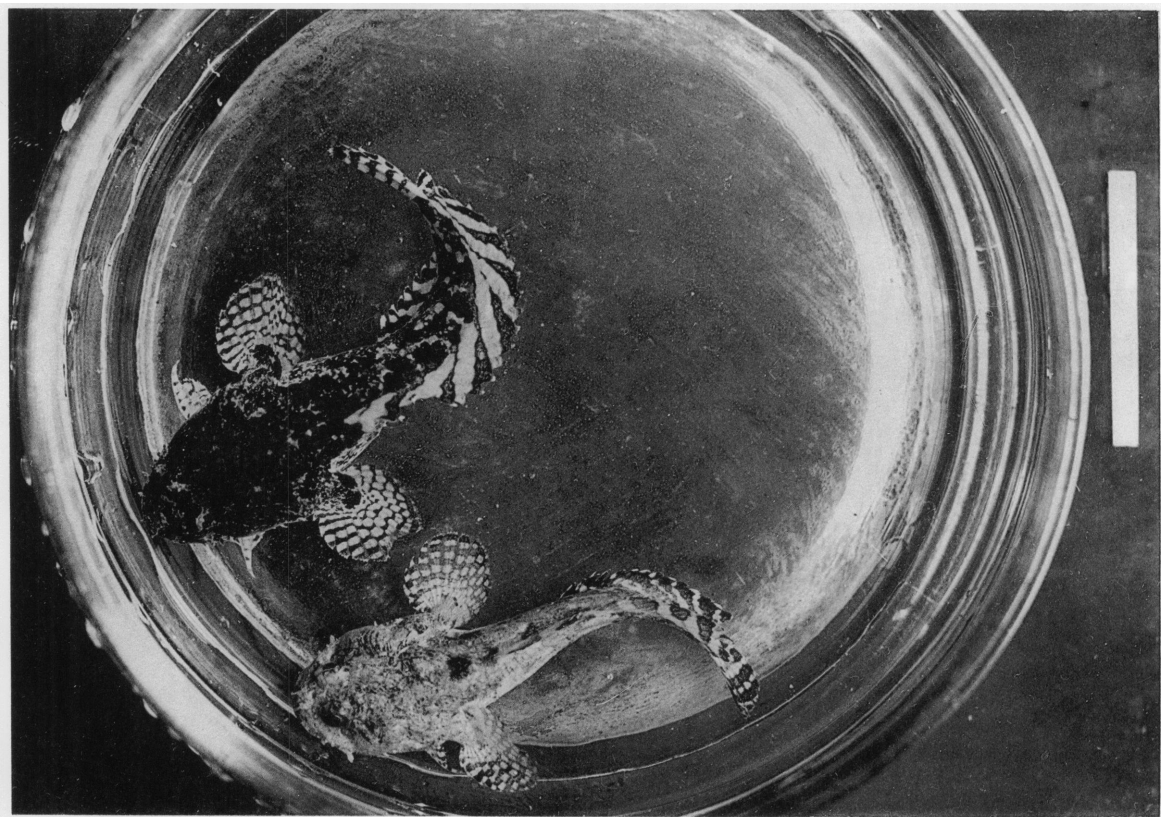




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1. Two conch fish (*Apogonichthys stellatus*), showing results of adrenalin injection in fish on left. All melanophores were concentrated by adrenalin

2. Two gobies (*Bathygobius soporator*), showing concentration of all melanophores in fish on right as a result of adrenalin injection

3. Two toad fish (*Opsanus* sp.), showing concentration of melanophores in fish on right as a result of adrenalin injection







