THE HISTOLOGICAL BASIS OF ADAPTIVE SHADES AND COLORS IN THE FLOUNDER PARALICHTHYS ALBIGUTTUS

By Albert Kuntz, Ph. D.
St. Louis University, School of Medicine

Contribution from the United States Fisheries Biological Station, Beaufort, N. C., and the Anatomical Laboratories of the St. Louis University School of Medicine

CONTENTS.

THE HISTOLOGICAL BASIS OF ADAPTIVE SHADES AND COLORS IN THE FLOUNDER PARALICHTHYS ALBIGUTTUS.

By ALBERT KUNTZ, PH. D.

Contribution from the United States Fisheries Biological Station, Beaufort, N. C., and the Anatomical Laboratories of the St. Louis University School of Medicine.

INTRODUCTION.

Changes in shade, color, and pattern which, as is well known, occur in many bony fishes are due primarily to changes in the distribution of the pigment granules in the chromatophores in the skin and changes in the relationships of the guanophores (cells containing guanin crystals) with these chromatic organs.

The present investigation [a] is an attempt to determine as accurately as possible the behavior of the chromatophores and other elements involved in the production of color in the skin of the flounder *Paralichthys albiguttus*, as it undergoes changes in shade or color or simulates as nearly as possible the color of a given background.

MATERIAL AND METHODS.

The material used in this investigation consisted of a large number of specimens of *Paralichthys albiguttus* ranging from 10 to 30 cm. in length. Many of these specimens were kept in aquaria of chosen colors continuously for three weeks or longer, others for briefer intervals.

Observations were made largely on living and fresh material. Sections of preserved skin were used primarily for the study of the distribution of pigment in the deeper layers of the skin and the relationships of the color producing elements with each other and with other structural elements in the skin in a vertical plane.

The chromatophores and guanophores lying immediately beneath the epidermis can be observed very satisfactorily on detached scales on which the epidermis remains intact. The removal of a few scales, if done carefully, causes little or no reaction on the part of the fish, and, consequently, no changes in the distribution of the pigment in the chromatophores. If these scales are mounted in sea water, no changes in the distribution of pigment granules occur for a considerable interval. When preparations of the whole skin were desired, the fish was killed instantaneously by chopping off the head

[a] This investigation was undertaken at the suggestion of Dr. S. O. Mast as a sequel to his work on "Changes in shade, color, and pattern in fishes, and their bearing on the problems of adaptation and behavior, with especial reference to the flounders *Paralichthys and Ancylopsetta*." Bulletin of the United States Bureau of Fisheries, vol. XXXIV, 1914, p. 176–238. *Paralichthys albiguttus* was selected because it was found by Dr. Mast to be highly adaptable to backgrounds of various shades and colors and was used extensively in his work. The author is indebted to Dr. Mast, also, for helpful suggestions and reading the manuscript.

with a single blow. Fish killed in this manner usually undergo no observable changes in shade or color for some little time. Preparations of the skin studied immediately after this operation show essentially the same distribution of the pigment granules in the chromatophores lying immediately beneath the epidermis as do the scales removed from the living fish. There is, therefore, probably no immediate change in any of the chromatophores in the skin. The chromatophores and guanophores in the deeper layers of the skin were studied in sections and also directly in pieces of skin from which some of the scales had been removed.

Skin desired for sectioning was fixed in Zenker's fluid, dehydrated as rapidly as possible, and immediately embedded in paraffin. The melanin pigment being very stable is not dissolved nor does it undergo any observable change in distribution during this process. The xanthine pigment being readily soluble in alcohol is completely dissolved. In the deeper layers of the skin the xanthophores can still be observed in sections; in the superficial layers they can not be distinguished from the guanophores which are closely associated with them. The guanophores remain intact and can be observed quite satisfactorily.

Skin desired for the study of the nerve supply to the chromatophores was prepared by the pyridine-silver method.

STRUCTURE AND DISTRIBUTION OF CHROMATOPHORES AND GUANOPHORES.

The skin of *Paralichthys albiguttus* contains chromatophores of two distinct types, viz, melanophores and xanthophores. The former contain melanin granules which vary in color from dark brown to black; the latter contain xanthine granules which vary in color from yellow to orange. Chromatophores of both of these types occur as isolated chromatic organs. Xanthophores are in many instances partly covered by overlying melanophores; however, chromatophore combinations involving a xanthophore and a melanophore, as described by Ballowitz (1913) in several species of teleosts, probably do not occur in this species. The melanophores are more numerous than the xanthophores. The averages of a large number of actual counts of the melanophores and xanthophores, respectively, in a given unit area in the superficial layer of the skin in nine specimens indicate a numerical ratio of melanophores to xanthophores in the layer immediately beneath the epidermis of 32 to 19.

A chromatophore consists essentially of a group comprising several or many pigment cells so arranged that the entire body presents a central area from which elongated processes radiate. In *Paralichthys* the melanophores are more nearly symmetrical and show a larger number of radial processes than the xanthophores.

A chromatophore is said to be "contracted" when the pigment granules are concentrated in the central area and "expanded" when the pigment granules are widely distributed. The terms "contracted" and "expanded" will be used in this paper in the sense here indicated and not with reference to the state of retraction or extension of the radial processes of the chromatophores.

A fully expanded melanophore (fig. 1, pl. 1) usually presents a relatively pigment-free central area bounded by a pigmented zone from which elongated pigmented processes radiate. Within the radial processes the pigment granules are usually disposed along more or less definite radial lines. Not infrequently radial processes show a dendritic structure; in other instances they are distinctly broader distally than proximally.

In a fully contracted melanophore all the pigment is concentrated in the central area. A fully expanded xanthophore (fig. 2, pl. 1) may or may not show a pigment-free area at the center. The central pigmented area is usually more or less irregular in outline and from it relatively few irregular processes radiate. The pigment granules show no regular arrangement. Xanthine granules disposed along definite radial lines are rarely observed. In a fully contraced xanthophore the pigment is all concentrated in a more or less irregular mass in the central area. Both melanophores and xanthophores can be observed in any phase intermediate between maximal expansion and maximal contraction.

Guanóphores are cells or groups of cells which contain guanin in the form of minute crystals. They occur either isolated or in groups and are frequently associated, more or less intimately, with chromatophores. Guanophores are sufficiently abundant in the superficial layers of the skin to occupy a relatively large part of the surface area. They also possess the capacity to contract and expand to a certain degree. When a fish changes from a darker to a lighter shade more or less rapidly, the guanophores occupy less of the surface area and appear somewhat more compact in the latter than in the former condition. The number of guanophores in the superficial layer of the

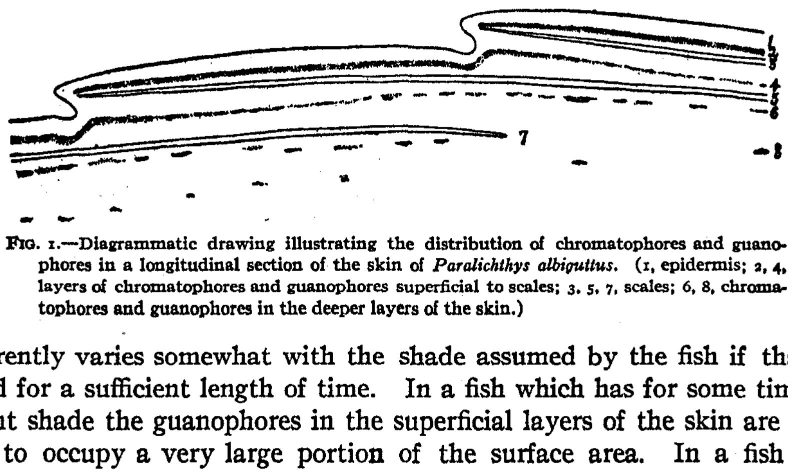

FIG. 1.—Diagrammatic drawing illustrating the distribution of chromatophores and guanophores in a longitudinal section of the skin of *Paralichthys albiguttus*. (1, epidermis; 2, 4, layers of chromatophores and guanophores superficial to scales; 3, 5, 7, scales; 6, 8, chromatophores and guanophores in the deeper layers of the skin.)

skin apparently varies somewhat with the shade assumed by the fish if that shade is maintained for a sufficient length of time. In a fish which has for some time assumed a very light shade the guanophores in the superficial layers of the skin are sufficiently abundant to occupy a very large portion of the surface area. In a fish which has for some time assumed a darker shade the guanophores in the superficial layers of the skin are apparently less abundant. Conclusive evidence on this point could not be obtained. Attempts at counting the guanophores in a given unit area are unsatisfactory because individual guanophores can not always be recognized. On the other hand, the degree of expansion of these bodies and their particular spacial relationships with the chromatophores are important factors in determining how large a portion of the surface area shall be occupied by them. Relatively few guanophores occur in the deeper layers of the skin.

The chromatophores and guanophores in the superficial layers of the skin are located superficial to the scales. The corresponding layers of these bodies, therefore, imbricate with each other as do the scales (text fig. 1). Both chromatophores and guanophores are more closely aggregated over the distal than over the proximal area of the scales. Consequently, they are most numerous over the area of each scale which is intimately covered by epidermis. Furthermore, the chromatophores in these areas differ somewhat in size and form from those nearer the proximal ends of the scales. Those lying superficial to the proximal areas of the scales and, consequently, beneath the overlapping areas of other scales, are characterized by relatively few irregular long and slender radial processes. Over the distal areas of the scales the majority of the melanophores lie immediately beneath the epidermis. In these areas, as well as over the proximal areas of the scales, the xanthophores lie somewhat deeper than the melanophores. Many of the guanophores lie at the level of the melanophores, while others lie somewhat deeper.

Relatively few chromatophores occur in the deeper layers of the skin. In many instances they are closely aggregated around blood vessels in the corium and in the subcutaneous tissue.[a] However, these chromatic organs in the deeper layers of the skin are probably too few and too far removed from the surface to play any important part in the shade or color assumed by the fish.

DEVELOPMENT AND DEGENERATION OF CHROMATOPHORES.

In surface preparations, as well as in sections of the skin, distorted and fragmented melanophores are not infrequently observed between the cells of the epidermis (text fig. 2), sometimes actually protruding from the surface. Doubtless these melanophores are being eliminated through the epidermis. This phenomenon was observed in the majority of the specimens examined, regardless of their shade or color. It was quite as common in specimens which were newly taken as in those which had been kept in aquaria for shorter or longer periods. Obviously the elimination of melanin pigment, and also its more or less continuous production, must be looked upon as normal physiological processes. A similar elimination of xanthine pigment through the epidermis was not observed. However, this negative observation affords no evidence that xanthine pigment is not eliminated in this manner. Xanthine pigment is highly unstable and would soon disintegrate following the fragmentation of the xanthophores.

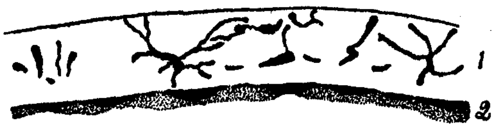

FIG. 2.—Camera lucida drawing showing fragmented melanophores between the cells of the epidermis. (1, epidermis; 2, layer of chromatophores and guanophores just beneath the epidermis.)

When a fish undergoes a change from a darker to a lighter shade and maintains that shade more or less constantly, a material reduction in the number of melanophores in the layer just beneath the epidermis takes place. Averages of a large number of actual counts of the melanophores in this layer in a given unit area of the skin of specimens newly taken or adapted to dark backgrounds and of specimens adapted to a white background indicate a reduction in the latter of approximately 30 per cent in the number of melanophores in the superficial layer of the skin. The rate at which this reduction took place in a single specimen which was placed on a white background as soon as it was brought into the laboratory is indicated by the curve *ab* in the accompanying figure (text fig. 3). This curve is based on the averages of a large number of actual counts of the melanophores in a given unit area on the surface of detached scales taken from various parts of the body. The figures in the vertical line indicate the number of melanophores in the given unit area, those in the horizontal line indicate the number of days following the initial counts. The initial counts were made when the fish was placed on the white background. This curve shows, furthermore, that a maximum reduction of approximately 28 per cent was reached in 11 days and that following this period the number of melanophores in the superficial layer of the skin remained fairly constant while the fish remained on the white background.

The averages of counts made on specimens which had been kept on a white background for a period of two months show, approximately, the same number of melanophores per unit area in the layer just beneath the epidermis as was shown by the counts made on the specimen used in the above experiment after it had been on the white background for a period of 11 days. It is probable, therefore, that when a fish becomes adapted to a background of a very light shade a maximum reduction in the number of melanophores in the superficial layer of the skin takes place in a relatively short time, and that little or no further reduction occurs even though a light shade is maintained for a prolonged period. This conclusion is supported by observations made by H. S. Willis on specimens which had been kept on given backgrounds for six months or longer.[a] This observer made camera lucida drawings under low magnification including all the chromatophores in the layer just beneath the epidermis on detached scales taken from various parts of the body. The averages of actual counts of the number of melanophores per unit area in these drawings indicate that melanophores are approximately equally abundant in the superficial layer of the skin of specimens adapted to black, blue, and red backgrounds and also of specimens adapted to white and yellow backgrounds. However, the average number per unit area in the drawings taken from specimens adapted to white and yellow backgrounds is only, approximately, 70 per cent of the average number per unit area in those taken from specimens adapted to black, blue, and red backgrounds. The difference here indicated in the number of melanophores per unit area in the superficial layer of the skin of specimens which had assumed dark and light shades, respectively, for a relatively long period is not appreciably greater than the corresponding difference indicated by the observations recorded above on specimens which had assumed dark and light shades, respectively, for relatively short periods.

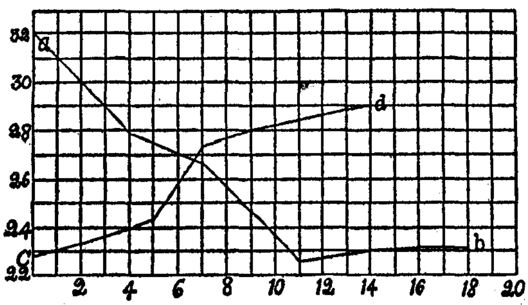

FIG. 3.—Curves showing the rate of change in the number of melanophores in the layer just beneath the epidermis. (For explanation, see text.)

When a fish in which the number of melanophores in the superficial layer of the skin has become reduced in response to the continued stimulus of a light background is transferred to a dark background, the number of melanophores in the superficial layer of the skin is gradually restored. The rate at which this restoration took place in a single specimen is indicated by the curve *Cd* in the accompanying figure (text fig. 3). This specimen was kept on a white background for four weeks and then transferred to a black background. The curve is based on the averages of 10 actual counts of the melanophores in a given unit area on detached scales taken from various parts of the body. Counts were made when the fish was transferred from the white background and at intervals of two or three days following this date. At the end of two weeks, when the experiment was discontinued, the number of melanophores per unit area was still somewhat less than the average for dark-colored specimens.

[a] These observations were made under the direction of Dr. S. O. Mast and are used here with his consent.

CHANGES IN DISTRIBUTION OF PIGMENT IN CHROMATOPHORES.

Changes in the distribution of the pigment granules in chromatophores have been studied by many investigators in both invertebrate and vertebrate animals. Nevertheless, the manner in which these changes are brought about remains a controverted question. Two theories prevail at present. According to the one, the chromatophores contract and expand in an ameboid manner. Among the supporters of this theory may be mentioned von Wittich (1854), Busch (1856), Leydig (1857, 1873), Hering and Hoyer (1869), Pouchet (1876), Halpern (1891), Ehrmann (1892), Ficabli (1896), Verworn (1909), Fröelich (1910), Holmes (1913), and Hooker (1912, 1914). According to the other theory, changes in the distribution of the pigment do not involve essential changes in the form of the chromatophore, but the pigment granules move through the protoplasm or in fixed canals in it. Among the supporters of this theory may be mentioned Brücke (1852), Harless (1854), Virchow (1854), Lister (1858), Solger (1889), Biedermann (1892), Zimmermann (1893), Keeble and Gamble (1905), Kahn and Lieben (1907), Winkler (1910), Degner (1912), Ballowitz (1893, 1912, 1913, 1914), Franz (1908, 1910), and Spaeth (1913).

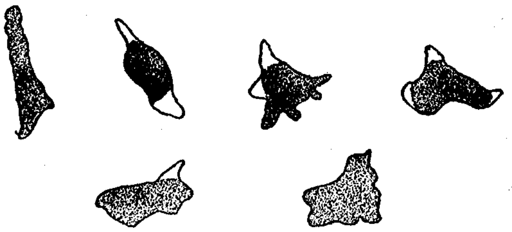

FIG. 4.—Camera lucida drawings taken at approximately 10-minute intervals of a pigment cell in an embroyo of *Lucania parva* 40 hours after fertilization. The stippled areas show the distribution of the pigment.

That it is quite impossible in fresh preparations of the skin or in living fishes to observe the limits of the processes radiating from the central area of a mature chromatophore except in its fully expanded condition is the common experience of not a few observers. That individual chromatophores possess a more or less definite form and that this form is retained, i. e., the position of each radial process remains apparently fixed, after repeated so-called "contractions" and "expansions" has been observed by many investigators. Therefore, if the processes radiating from the central area of a chromatophore are in turn retracted and extended, they must be composed of relatively fluid protoplasm and occupy more or less definite tissue spaces. That such is the case is maintained especially by Fröelich (1910) and Hooker (1914).

In isolated cells of the larvæ of *Hyla remolla* and *Diemyctylus torosus* containing black pigment Holmes (1913) observed unmistakable ameboid movements. While he admits the possibility that in the adult the cell processes of the chromatophores may be more fixed in outline, he still maintains that the extent to which ameboid movements occur in the pigment cells of larvæ ought to suggest skepticism as to the commonly accepted view that the pigment moves within the cell.

That ameboid movement occurs in the pigment cells of larval fishes is well known. The accompanying figure (text fig. 4) shows a series of camera lucida drawings taken at approximately 10-minute intervals of a cell containing yellow pigment in an embryo of *Lucania parva* 40 hours after fertilization.

The unshaded areas in these drawings illustrate areas free from pigment. The behavior of the pigment in the early pigment cells in embryos of this species is described by the writer as follows:

> The pigment granules arise in the central region of the cell and gradually push out toward the periphery. Until pigment is present in all parts of the cell the parts free from pigment remain clear.

Even after pigment has been present in all parts of the cell it may become concentrated in the central region leaving the peripheral region clear. In many instances as the pigment becomes concentrated isolated granules or groups of granules remain far out in the protoplasmic processes. The concentration and redistribution of pigment granules are obviously not due to ameboid movements of the cell, but to movements of the pigment granules in the cytoplasm.[a]

This description applies both to the cells containing yellow and those containing black pigment. It is in full accord with the findings of Franz (1908) in larvæ of *Pleuronectes platessa.*

While pigment cells in fishes are distinctly ameboid during embryonic and larval development, changes in the distribution of the pigment within them are probably not due to the form changes of the cells alone. If this were the only factor involved, we should expect that after pigment is once present in all parts of the cell the entire cell would remain pigmented regardless of its changes in form. The outlines of these cells are easily observed in early larvæ and, as indicated above, pigment-free areas arise after pigment has been present in all parts of the cell. On the other hand, changes in the distribution of the pigment granules frequently occur when no changes in the form of the cell are taking place. Therefore, the conclusion that changes in the distribution of the pigment granules in the chromatophores of larval fishes are due to ameboid form changes in the cells alone is unwarranted.

The functional activities of chromatophores were studied in considerable detail by Dr. S. O. Mast and the author in living specimens of *Paralichthys albiguttus.*[b] This species lends itself admirably to work of this kind. Specimens 5 to 10 cm. in length were used which had been kept in aquaria for some time and had been repeatedly transferred from one background to another of a different shade or color. They were consequently accustomed to handling and responded very promptly to changes in the shade or color of the background. A compound microscope was submerged in an aquarium containing water deep enough to cover a small flounder resting on a glass plate on the stage. The flounder was orientated so that the microscope could be focused on chromatophores in the caudal fin. When a white cardboard was placed under the glass plate and beneath the head of the fish the latter assumed a whitish shade. When the white cardboard was replaced by a black one the fish very promptly assumed a very much darker shade. These changes in shade took place so rapidly that the movements of the pigment granules in the melanophores in the field of the microscope could be observed quite satisfactorily even under high magnification.

As the fish assumed a light shade in response to the white background, the pigment granules in many of the melanophores in the field of the microscope could be seen retreating from the peripheral portions of the radial processes along more or less definite radial lines and becoming concentrated in the central area. As the fish assumed a darker shade in response to the black background, the pigment granules in the same melanophores could be seen advancing peripherally apparently along the same radial lines. Occasionally as the pigment granules advanced peripherally or retreated toward the center the radial lines would break, leaving spaces free from pigment granules. These spaces remained open for a short time and then gradually became filled by advancing or retreating granules. No evidence of the retraction and extension of the

[a] Bulletin Bureau of Fisheries, vol. XXXIV, 1914, p. 419.

[b] These observations were made at the United States fisheries biological station, Beaufort, N. C., during the summer of 1914.

radial processes of chromatophores could be observed. Rythmical movements consisting of alternating short advances and retreats of the pigment granules lying nearest the peripheral ends of the radial processes of melanophores were observed in fish resting quietly on a background to which they were thoroughly adapted. Again, not infrequently movements of pigment granules resembling Brownian movements were observed at the peripheral ends of the pigment masses extending into the radial processes of moderately expanded melanophores in resting fish. Such movements are characterized by Ballowitz (1913) as "a remarkable dance."

Changes in the distribution of the pigment granules in the xanthophores take place in essentially the same manner as in the melanophores. In these chromatophores pigment granules could be observed advancing toward the periphery and again retreating, but not along well-defined radial lines. In general the movements of the xanthine granules are more sluggish and less regular than those of the melanin granules.

The above observations on the behavior of the chromatophores in living specimens of *Paralichthys albiguttus* as they undergo changes in shade or color lead us to conclude that changes in the distribution of the pigment granules in the chromatophores do not involve essential form changes in these bodies, but that the pigment granules advance peripherally and in turn retreat toward the central area while the form of the chromatophore remains essentially unchanged.

Further evidence which indicates that the radial processes of the chromatophores are not extended and retracted with each corresponding change in the distribution of pigment is afforded by the fact that not infrequently as the pigment becomes concentrated in the central area of the chromatophore aggregates of pigment granules remain far out in the radial processes. If the radial processes were actually retracted, all the pigment granules would necessarily be carried toward the center with them. It might be suggested that these aggregates of pigment granules which remain at or near the periphery break through the surface film of the cytoplasm as the radial processes are withdrawn and are again engulfed as the latter are extended. However, this seems highly improbable.

The observations on the changes in the distribution of the pigment granules in chromatophores recorded in the preceding paragraphs substantiate similar observations made by Ballowitz on chromatophores in various species of teleosts. Ballowitz (1913) has, furthermore, described very delicate radial canaliculi in the chromatophores within which, he maintains, the pigment granules advance and retreat. The writer must confess he has not been able to observe such canaliculi in the chromatophores of *Paralichthys albiguttus* in either living or fixed material. Furthermore, the lack of any regular arrangement of the pigment granules in the xanthophores speaks strongly against the occurrence of radial canaliculi in these bodies.

INNERVATION OF CHROMATOPHORES.

Another important question regarding the functional activity of chromatophores involves their relationship to the nervous system. The results of operations on the eyes and nerves of fishes by Pouchet (1876), Šečerov (1909, 1913), Sumner (1911), and others indicate that the stimuli which normally call forth changes in shade or color which involve widespread changes in the distribution of the pigment granules in the chromatophores are received through the eyes. The experimental work of Mast (1914) proves conclusively that this conclusion is correct. The work of Pouchet (1876), furthermore, indicates that the chromatophores are under the direct control of the sympathetic nervous system. Ballowitz (1893) described a dense network of nerve fibers, presumably sympathetic in character, surrounding the chromatophores in the skin of certain fishes. The experimental work of Frisch (1910) on trout and minnows tends to show that the nerve fibers which control the chromatophores pass out at a precise level from the spinal cord into the sympathetic trunks and pass with them anteriorly and posteriorly. Frisch claims, furthermore, to have demonstrated a special center in the anterior end of the medulla whose stimulus brings on a contraction of the chromatophores.

In sections of the skin of *Paralichthys albiguttus* prepared by the pyridine-silver method the writer observed both medullated and nonmedullated nerve fibers which vary greatly in caliber. The medullated and the larger nonmedullated fibers were never observed in relationship with the chromatophores. However, many of the smaller nonmedullated fibers could be traced to their terminations on chromatophores (text fig. 5). These fibers do not form a dense network about chromatophores, as described and illustrated by Ballowitz (1893), but run more or less directly from the fiber bundles in the skin to their termination on the chromatic organs. The nonmedullated character of these fibers does not prove them to be sympathetic. However, this character, together with the small caliber of the fibers, strongly suggests that they are sympathetic in nature.

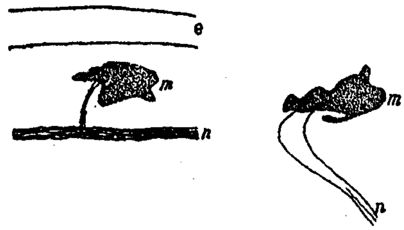

FIG. 5.—Camera lucida drawings of melanophores and nerve fibers associated with them in a section of the skin of *Paralichthys albiguttus* prepared by the pyridine-silver method. (*e*, epidermis; *m*, melanophores; *n*, nerve fibers.)

DETAILED STUDY OF CHROMATOPHORES AND GUANOPHORES IN SKIN OF FISH ON GIVEN BACKGROUNDS.

INTRODUCTION.

The experimental work of Mast proves conclusively that in members of the genus *Paralichthys* the skin simulates the background in color as well as in shade and pattern. In his summary he says:

> The range of changes in the skin in members of this genus is most remarkable. On a white background they become almost pure white, on a black background nearly black, and on gray backgrounds of various shades they become gray of very nearly the same shade. On blue, green, yellow, orange, pink, or brown of various hues they assume a color remarkably similar to that of the background. Reds of various tints and shades, however, are not very accurately simulated, but the color produced in the skin by each tint or shade of red is different from that produced by any other color and very different from that produced by gray regardless of the intensity.[a]

In the present investigation specimens of *Paralichthys albiguttus* were kept on backgrounds of given shades and colors until their skin simulated the shade or color of the background as nearly as possible. In general the shades and colors used were the same or similar to those used by Mast. The color-producing elements in the skin of these specimens, as well as of specimens newly taken, were studied microscopically in order to determine as accurately as possible what factors are involved in the production of given shades and colors.

[a] Bulletin Bureau of Fisheries, vol. XXXIV, 1914, p. 225.

FISH NEWLY TAKEN.

The specimens used in this study were taken on sandy beaches near the laboratory. The majority of them were brownish gray in color, mottled with areas of darker and lighter shades. Occasionally a specimen was taken which showed only a very slight or no brownish tone.

In the layer immediately beneath the epidermis in these specimens the melanophores in the darker areas were nearly or quite maximally expanded, while those in the lighter areas were expanded to a lesser degree. In the lightest areas many of the melanophores were contracted to such an extent that nearly all of the pigment was concentrated in the central area. The melanophores lay immediately beneath the epidermis and were not obscured by other structural elements.

In the majority of the specimens examined most of the xanthophores in the layer immediately beneath the epidermis were contracted to such an extent that the major part of the pigment was concentrated in the central area. From this central pigmented area irregular aggregates of pigment granules extended peripherally into the proximal portions of one or more radial processes. In this condition the pigmented area is so irregular in form that the radial arrangement of the parts of the chromatophore is not apparent. In those specimens which showed a marked brownish tone the xanthophores just beneath the epidermis were expanded to a moderate degree, while in those which showed little or no brownish tone they were almost maximally contracted. The xanthophores lay appreciably deeper than the melanophores, and many of them were partly covered by the latter. Many of them also were partly or completely overlain by guanophores.

The guanophores in the layer immediately beneath the epidermis occurred either isolated or in small groups, usually without any apparent arrangement which could be correlated with the arrangement of chromatophores or their component parts. However, in some instances guanophores were arranged in a radial manner about chromatophores with which they were intimately associated. Guanophores were present in sufficient numbers to occupy the greater part of the surface area beneath the melanophores as well as between them. They were apparently less abundant and somewhat more opaque in the darker than in the lighter areas.

Among the chromatophores lying superficial to the proximal areas of the scales and, consequently, beneath the overlapping areas of other scales the melanophores were well expanded while the xanthophores were expanded to an appreciably greater degree than the xanthophores in the layer just beneath the epidermis. Relatively few guanophores occurred superficial to the proximal areas of the scales. These also appeared less opaque than those in the layer just beneath the epidermis. The chromatophores in the deeper layers of the skin are well expanded.

Obviously the darker shades in the color pattern of a fish newly taken are due primarily to the degree of expansion of the melanophores in the darker areas. The lighter shades are due not only to the degree of contraction of the melanophores in the lighter areas but also to the effect of the guanophores, which to a large extent obscure the melanin pigment in these areas. In specimens which show a marked brownish tone this tone depends primarily upon the degree of expansion of the xanthophores. However, the quality of the color is not that of the xanthine pigment. It is probably the result of a blending of the colors of xanthine and melanin pigment plus the optical effect of the guanophores and other structural elements. In specimens which show only a very slight or no brownish tone the xanthophores are strongly contracted and so thoroughly obscured by the guanophores that the xanthine pigment has little or no effect.

FISH ON BLACK BACKGROUND.

The specimens used in this study were kept in a wooden aquarium, the bottom and sides of which were painted black. Some of them remained in this aquarium for 21 days or longer, others for shorter intervals. In those in which simulation of the background was most nearly perfect the skin was very dark gray with a very slight brownish tone, and the whole surface of the body was more uniform in shade than it was in the specimens newly taken.

In the layer just beneath the epidermis in these specimens the majority of the melanophores in the darker areas were maximally expanded. These melanophores showed a relatively large unpigmented area at the center, and pigment was present in the radial processes well toward their peripheral extremities. In the lighter areas the melanophores were expanded to a somewhat lesser degree. Many of them showed a relatively small unpigmented area at the center, while others showed no unpigmented central area but were still well expanded. In many areas the radial processes of adjacent melanophores interdigitated with each other. Where this condition obtained, a relatively large portion of the surface area was occupied by melanin pigment. Furthermore, the melanophores lay immediately beneath the epidermis and were not obscured by other elements.

The majority of the xanthophores in the layer just beneath the epidermis were strongly contracted. The portion of the surface area occupied by xanthine pigment was relatively small in proportion to that occupied by melanin pigment. Furthermore, the xanthophores lay appreciably deeper than the melanophores and many of them were wholly or in part overlain by guanophores. Occasionally a specimen which had become adapted to a black background was observed which showed scarcely a trace of brownish color. In these specimens the xanthophores in the superficial layer of the skin were contracted to a somewhat greater degree than usual and were more effectively obscured by overlying guanophores.

Guanophores were present in the superficial layers of the skin in approximately the same abundance as in specimens newly taken. In some instances they lay beneath melanophores. However, the majority of them lay in the areas not occupied by melanophores.

The chromatophores lying superficial to the proximal areas of the scales and in the deeper layers of the skin showed approximately the same degree of expansion as the corresponding chromatophores in the skin of the darker specimens newly taken.

The essential difference in the color-producing elements in the skin of specimens adapted to a black background and specimens newly taken from their natural environment consists in a markedly greater degree of expansion of the melanophores and a somewhat greater degree of contraction of the xanthophores in the layer just beneath the epidermis in the former than in the latter. The chromatic organs in the deeper layers of the skin show approximately identical conditions in both cases. The time required for a specimen taken from its natural environment to become maximally black is relatively short. Microscopic examination of the color-producing elements in the skin of specimens which had been on the black background two or three days showed conditions essentially identical with the conditions of the color-producing elements in the skin of specimens which had been kept on a black background continuously for a period of 21 days.

The accompanying figure (text fig. 6) is an attempt to illustrate the vertical distribution of chromatophores and guanophores in the layer just beneath the epidermis and in the layer just beneath this one overlying the proximal area of a scale in the skin of a specimen which was thoroughly adapted to a black background. The solid areas represent melanin pigment, the stippled areas include both xanthophores and guanophores. Figure 3, plate 1, represents a camera lucida drawing of chromatophores and guanophores in the layer just beneath the epidermis in the skin of a specimen 23 cm. in length which had been kept on a black background for a period of 21 days.

FISH ON WHITE BACKGROUND.

The specimens used in this study were kept for intervals varying from 5 to 25 days in a wooden aquarium the bottom and sides of which were painted white. Because of sediment in the water the bottom and sides of the aquarium assumed a brownish-yellow tinge. Consequently, the background to which these specimens became adapted was not pure white. When fully adapted to this background, the color of these specimens was grayish-white with a brownish-yellow tone, mottled with areas of a somewhat darker shade.

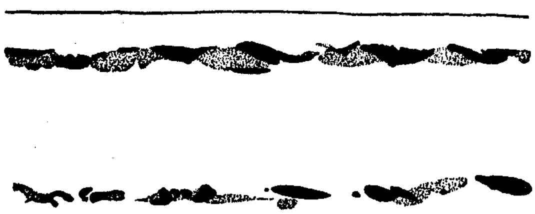

FIG. 6.—Camera lucida drawing illustrating the vertical distribution of chromatophores and guanophores just beneath the epidermis and superficial to the proximal area of a scale in the skin of a specimen of *Paralichthys albiguttus* adapted to a black background. Solid color represents melanin pigment, stippling includes both xanthophores and guanophores.

The melanophores in the layer just beneath the epidermis in the skin of these specimens were all contracted to a greater or lesser degree. In the lighter areas the majority of them were maximally contracted. In this condition the pigment was concentrated in the central area into a rounded mass, which usually lay somewhat deeper than the melanophores in their expanded condition. In areas of a somewhat darker shade the melanophores were contracted to a lesser degree. In these melanophores the major part of the pigment was concentrated in the central area, while masses of pigment granules extended for a short distance into the proximal portions of many of the radial processes or farther peripherally in a few radial processes. Melanophores occurred not infrequently, even in the lighter areas, in which the pigment was not appreciably concentrated. In these instances the central area of the melanophore lay at a deeper level than the peripheral portions and was deeply covered by guanophores. In the darkest areas many of the melanophores remained expanded to a moderate degree. In all these conditions the melanophores were obscured to a greater or lesser extent by associated guanophores.

The xanthopores in the layer just beneath the epidermis were contracted to a somewhat lesser degree than the corresponding xanthophores in the skin of specimens adapted to a black background, but were obscured to a greater extent by overlying guanophores. The majority of these xanthophores showed a heavily pigmented central area from which masses of pigment granules extended peripherally into the proximal portions of a few of the radial processes, giving the pigmented area a very irregular outline. In some instances the aggregates of pigment granules extended farther peripherally in one or two of the radial processes, while the others were entirely free from pigment. Relatively few xanthophores were expanded to a moderate degree and showed a more symmetrical distribution of the pigment granules. The ratio of the surface area occupied by xanthine pigment to that occupied by melanin pigment was appreciably greater in these specimens than in those adapted to a black background. However, the xanthophores were more effectively obscured by overlying guanophores in the former than in the latter.

Guanophores were apparently more numerous in the layer just beneath the epidermis in the skin of these specimens than in the skin of specimens newly taken. They occupied nearly all the surface area and were closely aggregated about many of the melanophores. As indicated in an earlier section of this paper, whether or not a real increase in the number of guanophores in the superficial layer of the skin occurs in specimens which become thoroughly adapted to a white background could not be definitely determined. Doubtless, the more complete occupation of the surface area by the guanophores in the skin of these specimens is due, at least in part, to the expansion of these bodies. Nevertheless, the changes which bring about the more complete occupation of the surface area by the guanophores constitute an important factor in the production of a still lighter shade following the initial response of the fish to a white background.

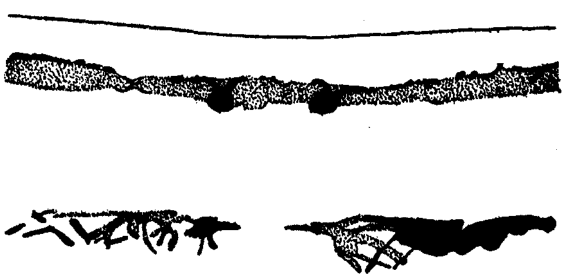

FIG. 7.—Camera lucida drawing illustrating the vertical distribution of chromatophores and guanophores just beneath the epidermis and superficial to the proximal area of a scale in the skin of a specimen of *Paralichthys albiguttus* adapted to a white background. Solid color represents melanin pigment, stippling includes both xanthophores and guanophores.

Both melanophores and xanthophores lying superficial to the proximal areas of the scales and in the deeper layers of the skin were contracted to a somewhat lesser degree, respectively, than those in the layer just beneath the epidermis. In many instances, while the radial processes could not be definitely outlined, the peripheral portions of many of them which contained no pigment granules still assumed a slight yellowish tinge.

The accompanying figure (text fig. 7) is an attempt to illustrate the vertical distribution of chromatophores and guanophores in the layer just beneath the epidermis and in the layer overlying the proximal area of a scale in the skin of a specimen which was thoroughly adapted to a white background. The solid areas represent melanin pigment, the stippled areas include both xanthophores and guanophores. Figure 4, plate 1, represents camera lucida drawings of chromatophores and guanophores in the layer just beneath the epidermis in a lighter and a darker area of the skin of a specimen 22 cm. in length which had been kept on a white background for a period of 25 days.

All the specimens which were kept in white aquaria became adapted to the white background very rapidly. The initial response to a white background consists in a marked concentration of the pigment granules in the majority of the melanophores and the depression of the central area of many of those in which the pigment does not become concentrated. This initial response may require but a few seconds and results in a distinctly whitish shade. However, a fish which has not previously been adapted to a white background does not become maximally white for a week or longer. Complete adaptation to a white background involves a rearrangement and probably an increase in the number of the guanophores and a material reduction in the number of melanophores in the layer just beneath the epidermis. On the other hand, a specimen which has recently been thoroughly adapted to a white background, but allowed to assume a darker shade for a short time, resumes a maximally white shade very promptly when again placed on a white background. The degree of concentration of the pigment granules in the chromatophores and the relationships of the guanophores with these bodies are essentially the same after the initial response in a specimen which has previously been thoroughly adapted to a white background as in one which has been on a white background continuously for a prolonged period.

The effect of a thorough adaptation to a white background remains apparent for a considerable length of time after the fish is placed on a dark background. This is shown by the following observations: A specimen 17 cm. in length which had been kept in a white aquarium for four weeks was placed, on August 2, in an aquarium painted black. This specimen assumed a somewhat darker shade almost immediately and gradually became darker. On August 4 it still remained somewhat lighter than the darker specimens newly taken. Many of the melanophores in the layer just beneath the epidermis were well expanded, but the central area still remained depressed and in many instances covered by guanophores. In some areas many of the melanophores remained almost maximally contracted while guanophores remained closely aggregated about them. In general, the melanophores were obscured to a lesser degree than when the fish was transferred from the white aquarium. On August 7 it had become very much darker, but not maximally black. In many areas the melanophores were maximally expanded, and their central areas were no longer depressed. In other areas they were expanded only to a moderate degree, while many of them still remained somewhat obscured by guanophores. The xanthophores remained in approximately the same condition as they were when the fish was transferred from the white aquarium. Guanophores still remained apparently more numerous in the superficial layer of the skin than in specimens which had not been adapted to a light background, but were somewhat contracted. Consequently, they occupied less of the surface area than when the fish still assumed a lighter shade. On August 12 this specimen appeared nearly as dark as other specimens in the same aquarium which were thoroughly adapted to the black background. The melanophores in the superficial layer of the skin now showed approximately the same degree of expansion as the corresponding melanophores in the skin of a specimen which was maximally black. Neither were they any longer obscured by overlying guanophores. These observations, in so far as they refer to the rate of adaptation, are in harmony with those presented by Mast.[a]

FISH ON YELLOW BACKGROUND.

The specimens used in this study were kept on a gamboge-yellow background for intervals varying from 5 to 26 days. When thoroughly adapted to this background, they were grayish yellow in color, mottled with areas of darker and lighter shades.

---

[a] Bureau of Fisheries, document no. 821, p. 195-199.

In these specimens the majority of the melanophores in the layer just beneath the epidermis were contracted only to a moderate degree. In the lighter areas the melanophores were contracted to a greater degree, but rarely was the pigment all concentrated in the central area. The melanophores lay immediately beneath the epidermis and were not obscured to an appreciable extent by overlying guanophores. However, the central area of those most fully expanded was not infrequently somewhat depressed.

Many of the xanthophores in the layer just beneath the epidermis were apparently maximally expanded, while others were expanded only to a moderate degree. In those which were not fully expanded the major portion of the pigment was concentrated in the central area, while masses of pigment granules extended toward the periphery but a short distance in some of the radial processes and farther in others. The pigmented area in these chromatophores was usually very irregular in outline. Rarely was a xanthophore observed in which all the pigment was concentrated in the central area. The ratio of the surface area occupied by xanthine granules to that occupied by melanin granules was relatively large. Because of the wider distribution of the pigment granules the color of individual xanthophores was less intense than in the skin of specimens not adapted to a yellow background.

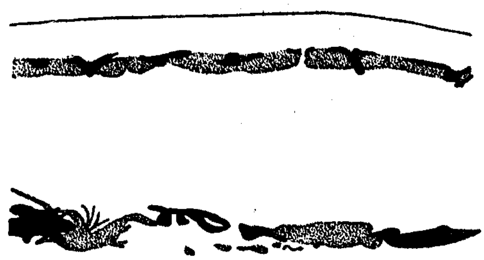

FIG. 8.—Camera lucida drawing illustrating the vertical distribution of chromatophores and guanophores just beneath the epidermis and superficial to the proximal area of a scale in the skin of a specimem of *Paralichthys albiguttus* adapted to a yellow background. Solid color represents melanin pigment, stippling includes both xanthophores and guanophores.

The guanophores in the layer just beneath the epidermis occupied a relatively large part of the surface area, rarely overlying melanophores, but very commonly overlying xanthophores, at least in part. However, the xanthine pigment was not largely obscured by them. Guanophores which lay above or in immediate contact with xanthophores assumed a greenish-yellow tinge. This phenomenon was more apparent in specimens adapted to a yellow background than in others, because the xanthine pigment in the former was more widely distributed. Guanophores not in close proximity with xanthine or melanin pigment in the skin of specimens adapted to a yellow background did not differ in shade or color from corresponding guanophores in the skin of specimens newly taken or those adapted to a dark background. The yellow color of the background had no direct effect upon the color of these bodies.

Both melanophores and xanthophores lying superficial to the proximal areas of the scales and in the deeper layers of the skin were well expanded, the xanthophores to a somewhat greater degree than the melanophores. Relatively few guanophores occur in these layers.

The accompanying figure (text fig. 8) is an attempt to illustrate as nearly as possible the vertical distribution of chromatophores and guanophores in the layer just beneath the epidermis and in the layer overlying the proximal area of the scales in the skin of a specimen which was thoroughly adapted to a yellow background. The solid areas represent melanin pigment, the stippled areas include both xanthophores and guanophores. Figure 5, plate 1, represents a camera lucida drawing of chromatophores and guanophores in the layer just beneath the epidermis in the skin of a specimen, 17 cm. in length, which had been kept on a yellow background for a period of 26 days.

All the specimens placed in a yellow aquarium became adapted to the yellow background almost as rapidly as those which were placed in a white aquarium did to the white background. The initial response of a dark-colored fish to a yellow background consists in a moderate degree of contraction of the melanophores and a marked degree of expansion of the xanthophores in the superficial layers of the skin. This initial response of a specimen which has not previously been adapted to a yellow background occurs somewhat less promptly than the initial response of a specimen newly taken to a white background. It results in a distinctly grayish-yellow color of a light shade. The production of a maximally yellow color requires no longer time than the production of a maximally white color. The complete adaptation to a yellow background involves some rearrangement of the guanophores and a moderate reduction in the number of melanophores in the layer just beneath the epidermis. The reduction in the number of melanophores is less marked than in the skin of specimens adapted to a white background. The averages of actual counts of the number of melanophores in a given unit area on detached scales taken from various parts of the body of specimens newly taken and of specimens thoroughly adapted to a yellow background, indicate a reduction in the number of melanophores in the layer just beneath the epidermis in the latter of approximately 16 per cent. No change in the number of xanthophores could be determined.

FISH ON GREEN BACKGROUND.

The specimens used in this study were kept, for intervals varying from 5 to 28 days, in a wooden aquarium the bottom and sides of which were painted moderately dark green. When thoroughly adapted to this background the general color of these specimens was a little lighter and more uniform than the color of the majority of the specimens newly taken and showed a distinct yellowish-green tone.

Many of the melanophores in the layer just beneath the epidermis in the skin of these specimens were nearly or quite maximally expanded while others were expanded only to a moderate degree. The condition of the melanophores in this layer did not differ materially from the condition of the corresponding bodies in the skin of many of the specimens newly taken in which the general color was most uniform.

The xanthophores in the layer just beneath the epidermis were expanded to a moderate degree. Many of them were in part overlain by guanophores, but were not largely obscured by the latter. The percentage of the total surface area occupied by xanthine pigment is appreciably greater in these specimens than in those adapted to either a black or a white background, but materially less than in those adapted to a yellow background.

The guanophores in the superficial layer of the skin occupied, approximately, the same percentage of the surface area as they did in the majority of the specimens newly taken. Guanophores which lay above or in immediate proximity with xanthine pigment assumed a greenish-yellow tinge. However, this was no more apparent in these specimens than in those adapted to a yellow background. The green background had no direct effect on either the shade or the color of the guanophores.

Superficial to the proximal areas of the scales and in the deeper layers of the skin both melanophores and xanthophores showed, approximately, the same degree of expansion as the corresponding chromatophores in the skin of specimens adapted to a moderately dark-gray background. Relatively few guanophores occurred in these layers.

Figure 6, plate II, is a camera lucida drawing of chromatophores and guanophores in the layer just beneath the epidermis in the skin of a specimen 19 cm. in length which had been kept on a green background continuously for a period of 28 days. The vertical distribution of chromatophores and guanophores in the skin of specimens adapted to a green background does not differ essentially from the vertical distribution of these elements in the skin of any moderately dark-colored specimen.

As a fish becomes adapted to a green background the melanophores in the superficial layer of the skin become expanded to approximately the same degree as in one adapted to a moderately dark-gray background. The xanthophores become expanded to a somewhat greater degree than the corresponding bodies in the skin of a specimen adapted to a gray background. That the guanophores in the superficial layer may assume somewhat different spacial relationships with the chromatophores is quite probable. However, no conclusive evidence on this point could be obtained. Greenish tones can not be observed microscopically except in the guanophores which lie in close proximity with xanthine pigment and in the almost pigment-free peripheral portions of the radial processes of some of the xanthophores. However, these greenish tones may be observed quite as well in the skin of specimens which have not been on a green background.

A microscopic study of the color-producing elements in the skin of these specimens affords no very definite data upon which to base an explanation of the cause of the greenish color which they assume. The most definite finding is the ratio of the surface area occupied by xanthine pigment to that occupied by melanin pigment. Doubtless, the greenish color is due to a mingling of the colors of xanthine and melanin pigment plus the optical effects due to the diffraction of light by the guanin crystals in the guanophores. The dark shade is due primarily to the degree of expansion of the melanophores.

FISH ON RED BACKGROUND.

Some of the specimens used in this study were kept for an interval of 21 days, others for a shorter interval, in a wooden aquarium, the bottom and sides of which were painted dark-carmine red. When thoroughly adapted to this background these specimens were reddish brown in color and of a shade approximately as dark as that of specimens adapted to a moderately dark-gray background.

The melanophores in the layer just beneath the epidermis in the skin of these specimens were all well expanded. In the darker areas the majority of them were nearly or quite maximally expanded; in the lighter areas they were expanded only to a moderate degree. In general, the melanophores in the superficial layer of the skin were expanded to a somewhat greater degree than in the majority of the specimens newly taken.

Among the xanthophores in the layer just beneath the epidermis, those which contain orange-colored pigment were well expanded, while those which contain yellow pigment were strongly contracted. In many areas the orange-colored xanthophores were nearly or quite maximally expanded; in others they were expanded only to a moderate degree. The majority of the yellow xanthophores were contracted to such a degree that the major part of the pigment was concentrated in the central area. Not infrequently xanthophores of this type were observed in which all the pigment was concentrated in the central area. Many of the xanthophores which were most strongly contracted were largely obscured by associated guanophores. Those which were expanded to a greater or lesser degree were not largely obscured by other elements except where they were in part overlain by melanophores. The ratio of the surface area occupied by xanthine pigment to that occupied by melanin pigment was greater than in specimens adapted to other dark-colored backgrounds, but appreciably less than in those adapted to a yellow background.

In general, the guanophores in the superficial layer were more intimately associated with the xanthophores than with the melanophores, and appeared to be arranged with respect to the former rather than the latter; consequently, they occupied a relatively large part of the surface area not occupied by melanophores. Inasmuch as a relatively large percentage of the guanophores lay in proximity with xanthophores, the greenish-yellow tone of the former, due to their proximity with xanthine pigment, was widespread and very apparent. The red background had no direct effect either on the shade or the color of the guanophores.

Superficial to the proximal areas of the scales and in the deeper layers of the skin the melanophores and orange-colored xanthophores were well expanded, while the yellow xanthophores were expanded only to a moderate degree. The peripheral pigment-free portions of many of the xanthophores, as well as the guanophores lying in proximity with xanthine pigment, assumed the same greenish-yellow tone as the guanophores lying in proximity with xanthine pigment in the superficial layer.

Figure 7, plate II, is a camera lucida drawing of chromatophores and guanophores as they appeared in the layer just beneath the epidermis in the skin of a specimen 20 cm. in length, which had been kept on a dark-red background for a period of 21 days. The vertical distribution of chromatophores and guanophores in the skin of specimens adapted to a dark-red background does not differ essentially from the vertical distribution of these elements in the skin of any dark-colored specimen.

Adaptation to a dark-red background involves a response on the part of the melanophores similar to the response of these organs to a moderately dark-gray background. The xanthophores whose color approximates the color of the background most closely, i. e., those containing orange-colored pigment, become greatly expanded. Consequently, the pigment which simulates the color of the background most closely becomes almost maximally effective. Furthermore, a relatively large percentage of the guanophores lie in proximity with xanthophores. Doubtless, the resulting reddish-brown color is due to a mingling of the colors of the xanthine and the melanin pigments plus the optical effects due primarily to those guanophores which lie in proximity with xanthophores. The dark shade is due to the degree of expansion of the melanophores.

Specimens newly taken which were placed in a red aquarium responded very promptly to the red background. The initial response involves a marked degree of expansion of the melanophores and orange-colored xanthophores and a moderate degree of contraction of the yellow xanthophores in the superficial layers of the skin.

It results in a color which approximates very closely the color of specimens which are thoroughly adapted to the same background. None of the specimens used in this study ever showed any color which approximated the red color of the background more closely than does the orange pigment.

FISH ON BLUE BACKGROUND.

The specimens used in this study were kept, for intervals varying from 5 to 22 days, in a wooden aquarium the bottom and sides of which were painted dark indigo-blue. Adaptation to this background was, perhaps, less perfect than it would have been to a blue background of a lighter shade. Those specimens which simulated the color of the background most closely were dark gray with a greenish-blue tone.

In these specimens many of the melanophores in the layer just beneath the epidermis were nearly or quite maximally expanded; others were expanded to a somewhat lesser degree. In general, the melanophores in this layer were expanded to a slightly lesser degree than in the skin of specimens adapted to a black background.

The xanthophores in the layer just beneath the epidermis were strongly contracted. In many areas the majority of them were contracted to a maximum degree. In others they were almost maximally contracted. In general the xanthophores lay somewhat deeper than when they are contracted to a lesser degree and were largely obscured by overlying guanophores.

The guanophores in the superficial layer were more definitely arranged with reference to the melanophores, and were apparently more intimately associated with these bodies than in any of the conditions described above. Not infrequently guanophores were closely aggregated around melanophores and extended well beneath the peripheral portions of the latter. Because so many of the guanophores were associated with melanophores in this manner there were relatively large areas between the melanophores not occupied by guanophores. Wherever guanophores were in close proximity with xanthine pigment they assumed a greenish-yellow tinge. However, relatively few guanophores were affected in this manner by reason of the relatively great degree of contraction of the xanthophores. On the other hand, many of the guanophores closely associated with melanophores and not in close proximity with xanthine pigment showed a greenish-blue metallic tone. The blue background had no apparent direct effect on the shade or the color of the guanophores.

Superficial to the proximal areas of the scales and in the deeper layers of the skin the melanophores were nearly or quite maximally expanded, while the xanthophores were strongly contracted. Many of the guanophores which were closely associated with melanophores showed the same greenish-blue metallic tone as was apparent in the superficial layer of the skin.

Figure 8, plate II, is a camera lucida drawing of chromatophores and guanophores as they appeared in the layer just beneath the epidermis in the skin of a specimen, 20 cm. in length, which had been kept on a dark-blue background for a period of 22 days. The vertical distribution of chromatophores and guanophores in the skin of specimens adapted to a dark-blue background does not differ essentially from the vertical distribution of these elements in the skin of any dark-colored specimen.

Adaptation to a dark-blue background involves almost maximum expansion of the melanophores and almost maximum contraction of the xanthophores in the superficial layers of the skin. The guanophores also undergo some changes in their relationships with the chromatophores, by which many of them become more intimately associated with melanophores, leaving relatively few intimately associated with xanthophores. Relatively few guanophores show a greenish-yellow tinge due to their proximity with xanthine pigment. On the other hand, many guanophores show a greenish-blue metallic tone, probably due to their intimate association and particular spacial relationships with well-expanded melanophores. Obviously the dark shade assumed by specimens adapted to a dark-blue background is due to the degree of expansion of the melanophores. The greenish-blue tone is probably due largely to the optical effect produced by the guanophores which are most intimately associated with melanophores.

DISCUSSION.

A comparative study of living material and preparations of the skin of specimens of *Paralichthys albiguttus* adapted to backgrounds of the various shades and colors used in this investigation indicates that shade depends primarily upon the degree of distribution of the melanin pigment in the melanophores and the spacial relationships of the guanophores with these bodies in the superficial layers of the skin. The xanthophores probably play no important part in the determination of shade. The melanophores in the deeper layers of the skin always respond less promptly and to a lesser degree to changes in the shade of the background than those in the superficial layer.

The most obvious response to a change in the color of the background is a change in the distribution of the xanthine granules in the xanthophores. Shades of yellow and orange probably depend primarily upon the relative degree of expansion of the xanthophores containing yellow and orange pigments. In general the particular quality of the color assumed depends on a complex group of factors which do not lend themselves readily to a detailed analysis. Some of the colors assumed may be duplicated by mixing pigments of the colors represented in the pigments contained in the chromatophores. Doubtless, these colors depend largely on the relative degree of expansion of the melanophores and xanthophores. Colors which can not be duplicated in this manner, doubtless, depend on the relative degree of expansion of the melanophores and xanthophores plus the optical effects due to the diffraction of light by the guanin crystals in the guanophores and possibly the absorption and diffraction of light by other structural elements in the skin. The optical effects produced by the guanin crystals are probably modified to some extent by the particular spacial relationships of the guanophores with the chromatic organs.

Obviously, certain colors are simulated much more perfectly than others. Among the colors used in this investigation yellow and green were simulated much more perfectly than dark red and dark blue. Autochrome plates published by Mast (1914) show that some of his specimens simulated the color of blue backgrounds much more perfectly than the specimens used in the present investigation. However, his specimens which were adapted to a red background did not simulate the color of the background any more closely than those adapted to a red background in this investigation. As indicated in an earlier section of this paper, none of the specimens placed on a dark-red background showed any color which approximated red more closely than the orange-colored pigment in the xanthophores. In view of these facts, the conclusion that all colors can be reproduced in the skin of the flounder is unwarranted.

SUMMARY.

1. Chromatophores in the skin of *Paralichthys albiguttus* are of two types, viz, melanophores and xanthophores. The former contain melanin granules which are dark brown or black, the latter contain xanthine granules which vary in color from yellow to orange. Changes in shade or color are due primarily to changes in the distribution of the pigment in the chromatophores and changes in the relationships of the guanophores with these bodies.

2. Many melanophores are eliminated from the skin through the epidermis. Under certain conditions the number of melanophores in the layer just beneath the epidermis becomes materially reduced.

3. Evidence is advanced in support of the theory that changes in the distribution of the pigment in the chromatophores are brought about by movements of the pigment granules toward the periphery and in turn back toward the central area while the form of the chromatophores remains essentially unchanged.

4. The evidence available indicates that the chromatophores are under the direct control of the sympathetic nervous system.

5. Shade is due primarily to the degree of expansion of the melanophores in the superficial layers of the skin. Very light shades maintained for a prolonged period involve a reduction in the number of melanophores in the superficial layer.

6. The initial response to a yellow background involves a moderate degree of contraction of the melanophores and a marked degree of expansion of the xanthophores in the superficial layers of the skin. Thorough adaptation to a yellow background involves a moderate reduction in the number of melanophores in these layers.

7. Adaptation to a green background involves approximately the same degree of expansion of the melanophores and a somewhat greater degree of expansion of the xanthophores in the superficial layers of the skin than adaptation to a moderately dark-gray background. The resultant yellowish-green tone probably depends upon the ratio of the distribution of the xanthine to the distribution of the melanin pigment plus the optical effect due to the diffraction of light by the guanin crystals in the guanophores.

8. Adaptation to a dark-red background involves almost maximum expansion of the melanophores and the xanthophores containing orange-colored pigment and a marked degree of contraction of the xanthophores containing yellow pigment in the superficial layers of the skin. The resulting reddish-brown color is due largely to the wide distribution of orange pigment, the effect of which is probably modified to some extent by the blending of orange and black and the optical effects produced by the guanophores. The dark shade is due to the wide distribution of melanin pigment.

9. Adaptation to a dark-blue background involves almost maximum expansion of the melanophores and almost maximum contraction of the xanthophores in the superficial layers of the skin. Many of the guanophores also become arranged with reference to the melanophores and closely associated with them. The xanthine pigment probably has little effect in the resultant color. The dark shade is due primarily to the wide distribution of the melanin pigment. Doubtless, the greenish-blue tone depends largely upon the optical effects produced by those guanophores which are closely associated with melanophores.

BIBLIOGRAPHY.

BALLOWITZ, E.

1893. Die Nervenendigungen der Pigmentzellen, ein Beitrag zur Kenntnis des Zusammenhanges der Endverzweigungen der Nerven mit dem Protoplasma der Zellen. Zeitschrift für wissenschaftliche Zoologie, bd. 56, p. 673–706; taf. XXXV–XXXIX. Leipzig.

1893a. Über die Bewegungserscheinungen der Pigmentzellen. Biologisches Centralblatt, bd. 13, p. 625–630. Leipzig.

1912. Über chromatische Organe in der Haut von Knochenfischen. Anatomischer Anzeiger bd. 42, p. 186–190, 2 taf. Jena.

1913. Die chromatischen Organe in der Haut von *Trachinus vipera* Cuv. Ein Beitrag zur Kenntnis der Chromatophoren-Vereinigungen bei Knochenfischen. Zeitschrift für wissenschaftliche Zoologie, bd. 104, p. 471–529, 5 taf. Leipzig.

1913a. Über schwarzrote und sternförmige Farbzellenkombinationen in der Haut von Gobiiden. Ein weiterer Beitrag zur Kenntnis der Chromatophoren und Chromatophoren-Vereinigungen bei Knochenfischen. Zeitschrift für wissenschaftliche Zoologie, bd. 106, p. 527–593, 5 taf. Leipzig.

1913b. Über Erythrophoren besonderer Art in der Haut von Knochenfischen. Archiv für mikroskopische Anatomie und Entwicklungsgeschichte, bd. 82, abt. 1, p. 206–219, 1 taf. Bonn.

1913c. Über schwarzrote Doppelzellen und andere eigenartige Vereinigungen heterochromer Farbstoffzellen bei Knochenfischen. Anatomischer Anzeiger, bd. 44, p. 81–91. Jena.

1913d. Das Verhalten der Zellkerne bei der Pigmentströmung in den Melanophoren der Knochenfische (Nach Beobachtungen am lebenden Objekt). Biologisches Centralblatt, bd. 33, p. 267–272. Leipzig.

1914. Vier Momentaufnahmen der intracellulären Pigmentströmungen in den Chromatophoren erwachsener Knochenfische. Archiv für Zellforschung, bd. 12, p. 553–557, 1 taf. Leipzig.

1914a. Zur Kenntnis des feineren Baues des Chromatophoren-Protoplasmas. Archiv für Zellforschung, bd. 12, p. 558–566. Leipzig.

BAUER, V., und DEGNER, E.

1913. Über die allgemein-physiologische Grundlage des Farbenwechsels bei dekapoden Krebsen. Zeitschrift für allgemeine Physiologie, bd. 15, p. 363–412, 35 text fig. Jena.

BIEDERMANN, W.

1892. Über den Farbenwechsel der Frösche. Pflüger's Archiv für Physiologie, bd. 51, p. 455–508. Bonn.

BRÜCKE, E.

1852. Untersuchungen über den Farbenwechsel des afrikanischen Chamäleons. Denkschriften der Kaiserlichen Akademie d. wissenschaften. Wien.

BUSCH, W.

1856. Phänomene aus dem Leben der Pigmentzellen. Arch. f. Anat., Physiol. und wiss. Med., p. 415–424.

DEGNER, E.

1912. Über Bau und Funktion der Krusterchromatophoren. Zeitschrift für wissenschaftliche Zoologie, bd. 102, p. 1–78. Leipzig.

EHRMANN, S.

1892. Beitrag zur Physiologie der Pigmentzellen nach Versuchen am Farbenwechsel der Amphibien. Archiv für Dermatologie u. Syphilis, bd. 24, p. 519–540. Wien.

FICABLI, E.

1896. Ricerche sulla struttura minuta della pelle degli Anfibî. Pelle degli Anuri della famiglea della Hylidæ. Atti Accad. Peloritana, Anno 11, Messina, 142 p., 2 fig. Messina.

FRANZ, V.

1908. Die Struktur der Pigmentzelle. Biologisches Centralblatt, bd. 28, p. 536–543, 545–548, 13 fig. Leipzig.

1910. Zur Struktur der Chromatophoren bei Krustaceen. Biologisches Centralblatt, bd. 30, p. 424–430. Leipzig.

FRISCH, KARL.

1911. Beiträge zur Physiologie der Pigmentzellen in der Fischhaut. Archiv für die gesammte Physiologie des Menschen und der Tiere, bd. 138, p. 319–387, 2 taf., 8 fig. Bonn.

1912. Über farbige Anpassung bei Fischen. Zoologische Jahrbücher, abt. für allgemeine Zoologie und Physiologie der Tiere, bd. 32, p. 171–230, 4 fig., 2 taf. Jena.

1912a. Sind die Fische farbenblind? Zoologische Jahrbücher, abt. für allgemeine Zoologie und Physiologie der Tiere, bd. 33, p. 107–126, fig. Jena.

1913. Weitere Untersuchungen über den Farbensinn der Fische. Zoologische Jahrbücher, abt. für allgemeine Zoologie und Physiologie der Tiere, bd. 34, p. 43–68. Jena.

FRÖHLICH, A.

1910. Farbenwechselreaktionen bei Palaemon. Archiv für Entwicklungsmechanik der Organismen, bd. 29, p. 432–438. Leipzig.

HALPERN, J.

1891. Über das Verhalten des Pigments in der Oberhaut des Menschen. Archiv für Dermatologie, u. Syphilis, bd. 23, p. 887–910. Leipzig.

HARLESS, E.

1854. Über die Chromatophoren des Frosches. Zeitschrift für wissenschaftliche Zoologie, bd. 5, p. 372–379. Leipzig.

HERING, T., and HOYER, H. F.

1869. Über die Bewegungen der sternförmigen Zellen und die dadurch erzeugten Veränderungen in der Hautfarbe der Frösche. Zentralblatt f. d. med. wiss., bd. 7, p. 49–54.

HOOKER, D.

1912. The reactions of melanophores of *Rana fusca* in the absence of nervous control. Zeitschrift für allgemeine Physiologie, bd. 14, p. 93–104, 3 fig. Jena.

1914. Amœboid movement in the corial melanophores of *Rana*. American Journal of Anatomy, vol. 16, no. 2, p. 237–250, 3 fig. Philadelphia.

KAHN, R. H., und LIEBEN, S.

1907. Über die scheinbaren Gestältanderungen der Pigmentzellen. Archiv. für Anatomie und Physiologie, Physioligische abt., p. 104–112, taf. 4, 5. Leipzig.

KEEBLE, F., and GAMBLE, F.

1905. The color physiology of higher Crustacea. Philosophical Transactions, Royal Society of London (Sr. B.), vol. 198, p. 1–16. London.

KUNTZ, ALBERT.

1916. Notes on the embryology and larval development of five species of teleostean fishes. Bulletin U. S. Bureau of Fisheries, vol. XXXIV, 1914, p. 407–429, 68 text fig. Washington.

LEYDIG, F.

1857. Lehrbuch der Histologie der Menschen und der Thiere. Hamm.

1873. Über die äusseren Bedeckungen der Reptilien und Amphibien. Archiv für mikrskopische Anatomie und Entwicklungsgeschichte, bd. 9, p. 753–795. Bonn.

LISTER, J.

1858. On the cutaneous pigmentary system of the frog. Philosophical transactions, Royal Society of London, vol. 148, p. 627–643. London.

MAST, S. O.

1916. Changes in shade, color, and pattern in fishes, and their bearing on the problems of adaptation and behavior, with especial reference to the flounders *Paralichthys* and *Ancylopsetta*. Bulletin U. S. Bureau of Fisheries, vol. XXXIV, 1914, p. 175–238, 3 text fig., 19 pl. Washington.

POUCHET, G.

1876. Les changements de coloration sous l'influence des nerfs. Journal de l'Anatomie et de la Physiologie, t. 12, p. 1–90, 113–165. Paris.

SEĆEROV, S.

1909. Farbenwechselversuche an der Bartzrundel (*Nemachilus barbatula* L.). Archiv für Entwicklungsmechanik der Organismen, bd. 28, p. 629–660, taf. 22, 23. Leipzig.

SOLGER, B.

1889. Zur Struktur der Pigmentzelle. Zoologischer Anzieger, bd. 12, p. 671–673. Leipzig.

1890. Über pigmentierte Zellen und deren Zentralmasse. Mitteil. d. naturwiss. Vereins v. Neu-Vorpommern und Rügen, 22 Jahrgang.

SPAETH, R. A.

1913. The physiology of the chromatophores of fishes. Journal Experimental Zoology, vol. XV, no. 4, p. 527–585, 3 text fig., 4 pl. Jena.

1913a. The mechanism of the contraction in the melanophores of fishes. Anatomischer Anzieger, bd. 44, p. 520–524, 3 fig. Jena.

SUMNER, F. B.

1911. The adjustment of flatfishes to various backgrounds: A study of adaptive color change. Journal Experimental Zoology, vol. 10, no. 4, p. 409–480, 13 pl. Philadelphia.

VERWORN, M.

1909. Allgemeine Physiologie, V. Auflage, 142 p., 319 abbild. Jena.

VIRCHOW, R.

1854. Chromatophoren beim Frosch. Archiv für pathologische, anatomie, und physiologie und für klinische medicin, bd. 6, p. 266–267. Berlin.

WINKLER, F.

1910. Beobachtungen über die Bewegungen der Pigmentzellen. Archiv für Dermatogie u. Syphilis, bd. 101, p. 255–260. Wien.

VON WITTICH, W.

1854. Die grüne Farbe der Haut unserer Frösche; ihre physiologischen und pathologischen Veränderungen. Archiv für Anatomie Physiologie u. wissenschaftliche Medicin, p. 41–59. Berlin.

ZIMMERMANN, K. W.

1893. Über die Contraktion der Pigmentzellen der Knochenfische. Verhandlungen der Anatomischen Gesellschaft, p. 76–78. Jena.

1893a. Studien uber Pigmentzellen. I. Über die Anordnung des Archiplasmas in der Pigmentzellen der Knockenfische. Archiv für mikroskopische Anatomie, bd. 41, p. 367–389. Bonn.

EXPLANATION OF PLATES.

PLATE I.

FIG. 1.—Expanded melanophore highly magnified.

FIG. 2.—Expanded xanthophore highly magnified.

FIG. 3.—Camera lucida drawing of chromatophores and guanophores just beneath the epidermis in a specimen of *Paralichthys albiguttus* adapted to a black background. × 550. The grayish tones represent guanophores.

FIG. 4.—Camera lucida drawing of chromatophores and guanophores just beneath the epidermis in two areas of the skin of a specimen of *Paralichthys albiguttus* adapted to a white background. × 550.

FIG. 5.—Camera lucida drawing of chromatophores and guanophores just beneath the epidermis in a specimen of *Paralichthys albiguttus* adapted to a yellow background. × 550.

PLATE II.

FIG. 6.—Camera lucida drawing of chromatophores and guanophores just beneath the epidermis in a specimen of *Paralichthys albiguttus* adapted to a green background. × 550.

FIG. 7.—Camera lucida drawing of chromatophores and guanophores just beneath the epidermis in a specimen of *Paralichthys albiguttus* adapted to a dark-carmine red background. × 330.

FIG. 8.—Camera lucida drawing of chromatophores and guanophores just beneath the epidermis in a specimen of *Paralichthys albiguttus* adapted to a dark-indigo blue background. × 550.

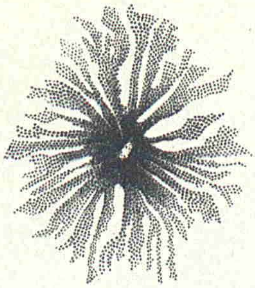

Fig. 1

Fig. 2

Fig. 4

Fig. 4

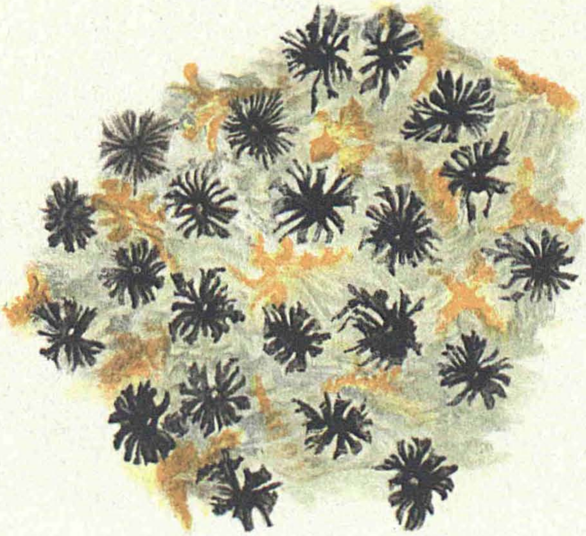

FIG. 7

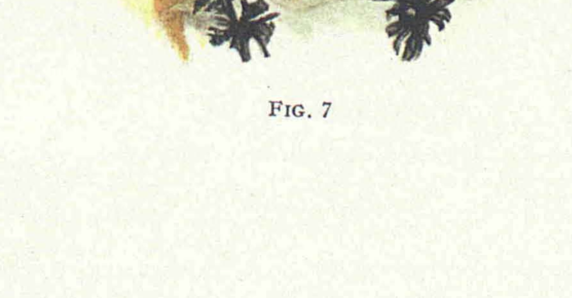

FIG. 7