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To

E. G. Conklin

With kindest regards from

G. H. Parker.

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UNIVERSITY OF PENNSYLVANIA

Leidy Memorial Lectures

COLOR CHANGES IN ANIMALS
IN RELATION TO NERVOUS ACTIVITY

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COLOR CHANGES OF ANIMALS IN RELATION TO NERVOUS ACTIVITY

By

G. H. Parker

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FOREWORD

DR. JOSEPH LEIDY was the first distinguished naturalist with whom I became acquainted. As a Jessup Student at the Academy of Natural Sciences in Philadelphia between the years 1880 and 1882 I was privileged to come under his direct supervision. At that time Dr. Leidy was Chairman of the Curators of the Academy and he very generously took upon himself a kindly oversight of the work of the Jessup beneficiaries. We spent half our day working upon certain assigned collections in the Academy and the other half upon the study of any subject that interested us. It was in these personal studies that Dr. Leidy was most helpful to us. His habit was to come about once a week to the Academy where he would spend part of the day either in the library or in his small private study. At such times he was always open to approach and we were free to bring to him any real difficulties that we had met in our work. To these he gave kindly consideration, and after such brief interviews we always left with renewed inspiration and encouragement. He knew us well enough to call us by our first names, a circumstance that put us in the very appropriate relation of apprentices to master.

As I look back on these brief contacts with Dr. Leidy I am surprised at what I unconsciously absorbed from them. I once sat on the outskirts of a group of young schoolchildren who had been invited by him to the Academy for a brief afternoon talk. He spoke to them on the human skull, a subject that at first sight might seem far from attractive to such youngsters, but before he had finished you could see the keen natural interest that

he had awakened in them for even so dry a topic. With his pencil he pointed out the chief apertures in the bony brain-case and told the children of their uses. When he came to the largest opening he called it by its technical name, the foramen magnum. He then remarked that this sounded very learned but he would warn them not to be overawed by such high-flown names and to remember that to an old Roman the Latin words merely meant a big hole. This simple experience in the use of scientific terms was a revelation to me and gave me a respect for the common English equivalents which I have never lost. A lesson of this kind meant more to me in my subsequent life as a teacher of zoölogy than pages of pedagogics.

Would that in the delivery of this discourse I could reawaken in you the childlike, lifelong enthusiasm that Dr. Leidy had for the study of Nature in all its fascinating aspects, and would that I could stir in you the generous impulses that in a second Dr. Leidy made these lectures possible.

G. H. PARKER

Harvard Biological Laboratories
March, 1936

PREFACE

THE present volume is a somewhat extended form of the Joseph Leidy Memorial Lecture in Science delivered at the University of Pennsylvania March 3, 1936. In addition to the historical summaries it consists in large part of the recent studies by my students and myself on the means of activating color-cells in the higher animals and on the significance of these processes for the workings of the nervous system. It is believed that the idea of the neurohumors, set forth in numerous earlier publications and rather fully elucidated in this volume, has a measure of truth in it for general nervous functions, and it is one of the objects of this essay to point out some of the reasons for accepting this idea and for testing its further applications. The whole proposal is quite obviously in a formative stage and, as every investigator knows, its outcome must await further study.

The invitation to deliver the Leidy lecture came to me from a committee consisting of Dr. Josiah H. Penniman, Provost of the University of Pennsylvania, Dr. Eliot Clark, Dr. Milton Greenman, and Dr. C. E. McClung. To these gentlemen I wish to express my keen appreciation of the honor of this invitation and the great pleasure I take in accepting it. A certain personal gratification that I feel in this acceptance I have attempted to indicate in the Foreword.

I cannot conclude this brief preface without acknowledging with sincere thanks the aid in preparing the manuscript for this volume received from my wife, Louise Merritt Parker, and from my assistant, Helen Porter Brower. I am also greatly indebted to Dr. F. M.

Carpenter of the Museum of Comparative Zoölogy for his care in the preparation of the illustrations as well as to the editors of the following publications for the privilege of reproducing the figures accredited to these sources: the *Journal of Experimental Zoölogy*, the *Biological Bulletin*, the *Journal of Experimental Biology*, the *Journal of General Physiology*, the *Proceedings of the National Academy of Sciences*, and the *Proceedings of the American Philosophical Society*.

The generous provisions for publication made by the Committee on the Leidy Memorial Lecture are very fully appreciated.

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I

INTRODUCTION

THE subject of color changes in animals was a familiar one to the ancients. Aristotle, in the second book of his *History of Animals*, declared that the chameleon, an inhabitant of the North Coast of Africa, can acquire either a black color, like that of the crocodile, or an ochereous one, like that of the lizard, or can be spotted with black like the panther. These changes, according to the Stagirite, take place over the whole body of this animal, for the eyes change like the rest and so does the tail. This description, together with certain other details recorded by Aristotle for this remarkable animal, was repeated almost verbatim by Pliny in the eighth book of his *Natural History*, to which he added the popular fiction that the chameleon feeds upon air. Pliny also recorded the color changes of the mullet, a Mediterranean fish much sought after as a delicacy for Roman feasts. In the ninth book of his *Natural History* he wrote that the masters of gastronomy inform us that the mullet while dying assumes a variety of colors and a succession of shades, and that the hue of its red scales growing paler and paler, gradually changes more especially if the fish is looked at enclosed in glass. Thus knowledge of these remarkable color changes was not only a part of ancient lore but was passed on to posterity. In *Henry the Sixth* Shakespeare put into the mouth of the infamous Duke of Gloucester the boast "I can add colors to the chameleon"; and no less a personage than Hamlet, when asked by his uncle-king how fares his health, replied "Excellent, i' faith; of the chameleon's dish: I eat the air." Thus did the Bard

of Avon play his part in transmitting truth and fancy about this interesting creature. It must be clear from these few allusions that the subject of this lecture has both the venerableness of age and the dignity of poetic association.

From very early times till about the beginning of the present century color changes in animals were believed by the majority of workers to be under the exclusive control of the nervous system. This opinion, often only vaguely and generally expressed, steadily gained ground in consequence of the accumulation of a large body of favorable evidence drawn in part from purely observational work and in part from experimental investigations. One outcome of these inquiries was to show that the eyes of animals are essential to their color changes, for when these organs were removed or effectually covered all signs of such changes disappeared, and the given animal so far as an alteration of its tint was concerned was largely incapacitated. In normal animals the color changes had long been recognized as means of harmonizing the creature with its surroundings. Even Aristotle in describing the habits of the common octopus remarked that this cephalopod would pursue any fish that came in its way, changing its color so as to imitate that of the neighboring rocks. This it also did when alarmed. In 1830 Stark, who had studied the color changes in a number of British river-fishes such as the perch, minnow, and the like, observed that when these fishes were on a light background they were pale in tone and when they were on a dark one they were of a deeper shade. He advanced the idea that this agreement, by which the fish was lost, so to speak, in its own background, was an advantage to it in its escape from enemies. This and other instances of a like kind gave rise to the modern theory of protective coloration, a system

of animal camouflage illustrated in the colors and forms of a great variety of organisms. From this standpoint the importance of the eyes in color responses became at once apparent, for an animal evidently must see that which it tends to resemble before it can assume the likeness. Thus color reactions became incorporated among the reflex activities of animals, and a wide and novel field for investigation was thrown open.

In 1858 the celebrated British physician Joseph Lister, then a student of medicine some thirty years of age, published in the *Transactions of the Royal Society of London* a scholarly paper on the color changes of the common frog. Here he summarized up to his own time the important general conclusions in this field of research. According to him the eyes of any animal that possessed the property of changing its tint were the only channels, to use his expression, through which the rays of light could gain access to the nervous system so as to induce changes of color in the skin. He declared further that the cerebro-spinal axis was chiefly, if not exclusively, concerned in regulating the functions of the pigment-cells. These brief statements give the physiological foundations upon which has been based the experimental work on animal coloration during the last half of the nineteenth century.

Incidentally these early investigations afforded a general survey of the animal kingdom so far as color changes were concerned. As an outcome of such an inspection it was found that these changes are limited in the main to comparatively few representatives of five important groups of the higher animals. These are the cephalopods such as the octopus, the cuttlefish, and the squid; the crustaceans, especially the shrimps and prawns; and, among the vertebrates, the fishes, the amphibians, and the lizards. The highest vertebrates, the birds and

mammals, with their coverings of feathers and of hairs almost entirely lack this capacity. Among these forms man, so far as I am aware, is the only species which in a feeble way keeps up this type of reaction. Our facial blush is dependent upon a temporary enlargement of the small blood-vessels of the skin and corresponds in all essential respects with the reddening of the integument seen in such fishes as the top-minnows. But even this mild activity, once such a powerful weapon in the hands of the female of the species, will, I fear, soon find its place among the lost arts, for the modern generation seems to have given up a reaction-pattern that was at once the charm and delight of an earlier day.

Another all-important step taken by these older workers was the discovery of the means by which color changes were brought about. Over a century ago it was found that those animals that show changes in tint possess in their integuments a multitude of minute bodies which by what appear to be contractions and expansions are able to lighten, darken, or otherwise alter the color of their possessors. These bodies were studied in the cephalopods in 1819 by the Italian naturalist Sangiovanni who called them *cromofori*, or in English chromatophores. It is now known that chromatophores are single integumentary cells or groups of such cells containing pigment which by one means or another may be concentrated and thus rendered inconspicuous, or may be spread out and thus become exposed to view. In the cephalopods, such as the octopus and the squid, each chromatophore consists of a central elastic-walled sac filled with pigment, around which is a system of radiating muscle-fibers (Fig. 1). By means of these fibers the spherical sac may be drawn out to a flattened disc, thus spreading its pigment conspicuously, or it may be allowed to contract to a minute sphere almost in-

FIG. 1. Chromatophores of the squid *Loligo*: *a*, contracted; *b*, expanded. Bozler, *Zeit. vergl. Physiol.*, 1928, 7, 381, fig. 1.

visible. In the crustaceans, especially the shrimps, the chromatophores (Fig. 2) are usually groups of cells which may carry each cell for itself a distinctive color. By internal migration these colors become variously dispersed or concentrated, thus adding or subtracting their share in the general color tone of the whole animal. Finally in the vertebrates the great majority of chromatophores (Fig. 3) are single cells of which there are several classes: melanophores, containing dark pigment granules; xanthophores and erythrophores carrying yellow, orange, or red carotenoid pigments; and finally,

FIG. 2. Chromatophore with fully dispersed pigment from the shrimp *Palaemonetes*. Perkins, *Jour. Exp. Zool.*, 1928, 50, 101, pl. 1.

FIG. 3. Group of chromatophores each with fully dispersed pigment from the tail of the killifish.

though this may not conclude the list, leucophores and iridocytes with their semi-crystalline or crystalline guanine-like contents. These various types of chromatophores, partly through their own innate color exposures and withdrawals, partly through their effects in covering and uncovering other colored cells, combined also with such physical light changes as are induced by the thin transparent outer layers of the skin, have united to produce that marvelous play of animal colors which once fed the eyes of the ancient Roman gourmands and which now drives the modern biologist almost to despair.

In 1852 Brücke published his important monograph on the color changes of the African chameleon. In this work he pointed out that when cutaneous nerves were cut the denervated area of the skin made itself manifest by darkening, that is, the dark pigment in the melanophores of this area became dispersed. Brücke expressed the natural opinion that nerves severed in this way had suffered paralysis and that the melanophores with which these nerves were connected, having been released from nervous control, lapsed into an inactive state. He therefore regarded the stage of a melanophore with dispersed pigment as the relaxed or resting one as contrasted with that of concentrated pigment which he believed to be the fully active stage. In this way he brought chromatophores into line with ordinary muscle fibers. This was a generalization of no small significance and has been accepted by most later workers. We shall see, however, that it may be open to question.

In the early seventies of the last century the French physiologist Pouchet (1872, 1876) carried out experiments upon fishes similar to those that Brücke had performed on chameleons. Pouchet cut integumentary nerves and noted, for instance in turbot (Fig. 4), that the denervated areas darkened as they had done in the

chameleon. Pouchet, however, showed still further that if the spinal cord of a fish is cut, no such integumentary darkening follows. This response took place only when the sympathetic chains situated one on either side of the vertebral column were severed. He therefore declared that the chromatophoral system was not only under the control of nerves but that these nerves were sympathetic in origin. This conclusion has been abundantly confirmed by a large number of investigators, among whom the chief is von Frisch (1910, 1911, 1912*a*, 1912*b*) whose studies on the color changes in fishes were published about the beginning of the second decade of this century and were a brilliant continuation of the masterly work of his predecessors.

But even before this time, as von Frisch himself recognized, a new current of ideas had set in. This resulted from a series of incidental observations the significance of which was not at first fully appreciated. In 1898 Corona and Moroni showed that when adrenalin, the secretion of certain cells in the medulla of the adrenal gland, was introduced into the circulation of a frog the pigment in its melanophores became strongly concentrated. This unique observation was confirmed by Lieben in 1906 who made an extended investigation of the subject. Comments by Fuchs in 1914 on these two

FIG. 4. Turbots in which particular nerves have been cut whereby the melanophores of the denervated regions have been induced to disperse their pigment thus rendering the fishes dark in those regions. Pouchet, *Four. Anat. Physiol.*, 1876, 12, pl. 4.

pieces of work led Redfield in 1918 to investigate the effects of this hormone on the chromatophores of the lizard *Phrynosoma* with the result that adrenalin was found also in this instance to be a potent agent in concentrating chromatophoral pigment. Redfield, after an exhaustive study of the color changes in *Phrynosoma*, expressed the opinion that the melanophores in this lizard were under the control of two types of agents, nervous and hormonal, and that both these agents in this particular instance were concerned with the concentration of the melanophore pigment, that is, with the blanching of the animal. Here then was evidence of a novel form of chromatophoral control, one in which hormones or, as these particular hormones are now called, neurohumors, are concerned.

In amphibians and crustaceans the process of nerve cutting as carried out by the older investigators had never yielded, even in the hands of the most skilful, conclusive and satisfactory results such as had been obtained from fishes and reptiles. It is therefore not surprising that skeptical investigators of this subject should turn their attention to amphibians and crustaceans with the view of ascertaining what can be learned from them as to the control of chromatophores. As Hogben (1924) remarks in his volume on the Pigmentary Effector System, this line of attack was especially suggested by the researches of Adler (1914), P. E. Smith (1916), and Allen (1917), who developed the technique of hypophysectomy in anuran larvae and called attention to the extreme pallor which comes over these young animals after the removal of the germs of their pituitary glands. These workers, however, did not appreciate clearly the full significance of such pigmentary changes; this was first pointed out by Atwell (1919). In 1921 Swingle noticed that tadpoles darkened when the intermediate

part of the pituitary gland was implanted in them. The blanching of the common frog after the removal of its pituitary gland was incidentally recorded by Krogh in 1922. Meanwhile Hogben and Winton had been actively engaged in experiments on the color changes of frogs in relation to nerves and the pituitary secretion. Their papers, which appeared in 1922 and 1923 and were based chiefly on a study of the European frogs, led to rather startling conclusions. In these it was pointed out that nerves played a wholly insignificant part in the control of color changes in amphibians, if, in fact, they had any part at all. The dark phase of the frog was shown to depend upon a secretion derived from the pituitary gland, probably from its intermediate part. This secretion was carried from the gland by the blood to the melanophores, whose pigment was thereby induced to disperse. The pale phase of the animal was not so consistently worked out. In the early steps in his work Hogben appears to have entertained the view that this single neurohumor, the intermediate-pituitary secretion, was all that was involved in the frog's change of color. This secretion when present in the fluids about the melanophores called forth the dark reaction, and its absence from these fluids allowed the pale phase to intervene. Subsequently Hogben and his associates worked upon other species of frogs and particularly upon the South African toad, *Xenopus*. As a result of these later investigations he and Slome became persuaded that at least in the amphibians just named there was evidence for a second humor which, though derived from the pituitary complex, nevertheless induced a concentration of melanophore pigment (Slome and Hogben, 1928, 1929; Hogben and Slome, 1931). This humor, though a product of the pituitary gland, was thus the counterpart of the first one. These investigations were pub-

lished a number of years after Hogben's earlier work, and though they add somewhat to the complexity of the picture of chromatophoral control in amphibians, they leave that picture essentially as it was originally outlined by Hogben himself: nerves relegated to the background, perhaps entirely excluded, and hormones the all-important factors. The contrast between this new way of conceiving the adjustment of amphibian chromatophores to environmental changes and that envisaged by the older workers is enormous. As was pointed out by Hogben in 1924, this view of color-cell activation sets off the amphibians in strong contrast with the fishes and the lizards, in both of which there appeared to be ample and complete evidence for the nervous control of their color-cells.

Quite independent of the work on amphibians, but emerging eventually in much the same way, is that done by recent investigators on crustaceans. Like the frogs and toads, shrimps and other crustaceans had never yielded in nerve-cutting experiments evidence favorable for a nervous interpretation of the control of their chromatophores. In 1925 Koller noticed that when the blood of a dark-tinted Crangon, a common Atlantic shrimp, was drawn and injected into a pale one the latter quickly grew dark. This at once suggested that in these animals neurohumors may be in the blood and may be the means of controlling the color-cells. This idea was followed up by Perkins who in 1928 published an account of the color changes in another Atlantic shrimp, *Palaemonetes*. Perkins was unable to repeat with success in this form the experiment on the transference of blood carried out by Koller on Crangon, but he nevertheless sought in the body of *Palaemonetes* for an organ that might produce a humor controlling the color-cells. This he finally found in the eye-stalks of

the shrimp. When a number of the eye-stalks of pale *Palaemonetes* were crushed and extracted with seawater a solution was obtained which on being injected with proper precautions into a dark shrimp of the same species caused the pigment in its chromatophores to concentrate and the shrimp to blanch. Perkins showed further that the cutting of nerves in *Palaemonetes* had no effect whatever upon its color changes, but that a temporary obstruction to the flow of blood in certain of its blood-vessels was followed by a very profound color change. These various observations have been repeated and confirmed by subsequent workers and with such a wealth of detail that we are now fully justified in concluding that among crustaceans, as among amphibians, nerves play no direct part in the control of chromatophores which at least in these groups of animals are under the exclusive influence of special hormones, the neurohumors. Such secretions are produced in some distant part of the body under excitation received from the eye and transported from their region of origin by the blood to the responding color-cells. Thus it appears that color changes may be controlled in animals through one or other of two radically different physiological systems: a direct nervous control as seen particularly in fishes and in reptiles, and a secretory or neurohumoral one as exemplified in crustaceans and amphibians. It is now proposed to examine these two types of control and to ask the question, are they as different as at first sight they appear to be, or have they elements enough in common to allow them to be brought under one general plan of action (Parker, 1932)? In attempting this analysis I shall discuss the color changes of two fishes, the common smooth dogfish, *Mustelus canis*, and the killifish, *Fundulus heteroclitus*, a small top-minnow of the Atlantic coast.

II

THE DOGFISH

THE first fish whose chromatophore system I wish to consider is the common smooth dogfish of the New England coast, *Mustelus canis*. This fish was shown first by Lundstrom and Bard (1932) to have a decided though limited color change. On a white background it grad-

FIG. 5. Two smooth dogfishes, *Mustelus*, originally of the same tint, twenty-four hours after the removal of the hypophysis from the fish on the right. They now show extreme differences in tint. Lundstrom and Bard, *Biol. Bull.*, 1932, 62, pl. 1.

ually and slowly becomes pearly white, often with a pinkish tint due to the color of the blood showing through its translucent skin. On a black background it darkens more quickly to a deep slate color (Fig. 5). As a microscopic inspection of its skin shows, the pale phase is due to a concentration of its melanophore pigment (Fig. 6) and the dark one to a dispersion of this coloring matter (Fig. 7).

The dark phase was very fully studied by Lundstrom and Bard. They showed that after the pituitary gland had been extirpated this fish began to blanch in about thirty minutes and reached full pallor in some twelve hours. This pallor was maintained even when the fish was kept on a black background where under ordinary circumstances it would have turned dark. The part of

the pituitary complex that was concerned with this color change was shown by Lundstrom and Bard to be the so-called posterior lobe, which in the dogfish probably includes the portion designated in the higher vertebrates as the *pars intermedia*. When an extract made from this lobe was injected into a pale hypophysectomized *Mustelus* a distinct general darkening of the fish occurred within three minutes, and after an hour the

FIG. 6. Dermal melanophores of a smooth dogfish, *Mustelus*, showing their pigment in extreme concentration causing the fish to appear pale. Lundstrom and Bard, *Biol. Bull.*, 1932, 62, pl. 4.

FIG. 7. Dermal melanophores of a smooth dogfish, *Mustelus*, showing their pigment in extreme dispersion causing the fish to appear dark. Lundstrom and Bard, *Biol. Bull.*, 1932, 62, pl. 3.

animal was found to be as deep in tint as were fully dark normal individuals. In from five to six hours thereafter the dogfish had returned to its original pale shade. Tests of the effects of fractions of the posterior lobe showed that an amount of extract that represented one twenty-fifth of this lobe would induce a noticeable local deepening of color, while that equal to one-tenth to one-seventh of a lobe would excite full darkening. Commercial preparations of the posterior pituitary lobe such

as "pituitrin" (Parke, Davis & Co.) and "infundin" (Burroughs, Wellcome & Co.) also brought on darkening in pale pituitaryless dogfishes. The melanophores in isolated pieces of dogfish skin reacted to these various solutions in the same way as did the color-cells in the whole fish. From these and other results Lundstrom and Bard concluded that the darkening of *Mustelus* was due to the action of a substance from the posterior lobe of the pituitary gland in dispersing the melanophore pigment which under unstimulated conditions was concentrated in the color cells. Thus these authors gave a very adequate account of the darkening of *Mustelus*, but passed over its pale phase with only slight consideration.

Two years later Parker and Porter (1934) repeated the essential parts of Lundstrom and Bard's work and obtained confirmatory results. They observed further that when the defibrinated blood from a dark dogfish was injected into a pale one, a dark area resulted showing that the blood, as might have been expected, carried a neurohumor that induced a dispersion of the melanophore pigment. (Incidentally it may be remarked that the defibrinated blood from a pale dogfish when injected into a dark one had no effect upon the tint of the recipient.) These observations all support the conclusion that the dark phase of *Mustelus* is due to a dispersing neurohumor produced in the pituitary gland and carried from that gland by the blood to the responding color-cells.

Parker and Porter, however, went further than to confirm the results of Lundstrom and Bard. They attempted to test for blanching in *Mustelus* by cutting its nerves. Whenever an integumentary nerve in a dark dogfish was cut the area thus denervated soon blanched. This was best seen in the fins. If a cut about one

centimeter long is made through a pectoral fin of a dark dogfish at right angles to the fin-rays and about a centimeter and a half from the edge of the fin, there will be produced a light band which, starting from the cut, will extend over the dark part of the fin to its pale edge (Fig. 8). The band will begin to appear in from ten to fifteen minutes after the cut has been made. It will reach its maximum in about a day, after which it will gradually disappear in from two to

FIG. 8. Dorsal view of a pectoral fin from a dark dogfish, *Mustelus*, showing a light band about an hour after the initiating transverse cut had been made in the fin. Parker and Porter, *Biol. Bull.*, 1934, 66, pl. 1, fig. 1.

FIG. 9. Dorsal view of a pectoral fin from a pale dogfish, *Mustelus*, showing a light band several days after the initiating transverse cut had been made. Parker and Porter, *Biol. Bull.*, 1934, 66, pl. 1, fig. 4.

three days. Light bands of this kind can be excited even in dogfishes in the pale phase, showing that the concentration of the melanophore pigment in such a band is more extreme than it is in normal pallor (Fig. 9). The pale bands produced by cutting are not the result of circulatory disturbances, for, as can be seen under the microscope, their areas even directly after the operation show an active and apparently normal circulation of blood. Moreover in many parts of the dogfish the nerves take very

different directions from the blood-vessels and when cuts are made in these regions the blanched bands

FIG. 10. Dorsal view of a pectoral fin from a dark dogfish, *Mustelus*, showing a light band in process of gradual disappearance two days after the initiating cut had been made. Parker and Porter, *Biol. Bull.*, 1934, 66, pl. 1, fig. 2.

band, no invasion will occur on that side but the band will gradually disappear by invasion from the opposite side and from that side only (Fig. 11). This condition suggests that the disappearance of pale bands results from the lateral infiltration of some darkening agent. At least there is no evidence of any other factor being involved, for a cut made in a pale fish is never under any circumstances followed by the formation of a dark band or any other such change.

follow the courses of the nerves and not those of the vascular supply.

When a pale band on a dark dogfish begins to disappear, it does so by lateral invasion. The dark area of the fin in general creeps in on the pale band from the two sides till the band is obliterated (Fig. 10). If, before this invasion of the pale band has set in, a longitudinal cut is made along one edge of the

FIG. 11. Dorsal view of a pectoral fin from a dark dogfish, *Mustelus*, showing a light band in process of disappearance, an operation here locally checked by a longitudinal cut on one side of the band. Parker and Porter, *Biol. Bull.*, 1934, 66, pl. 1, fig. 3.

That the pale bands in *Mustelus* are the result of nerve stimulation is rendered highly probable from the following experiment (Parker, 1935a). If two needle holes are made in the base of the anterior dorsal fin of *Mustelus*, one about five millimeters in advance of the other, and the two platinum electrodes of an induction apparatus are inserted one in each hole, the preparation after having been allowed to stand for half an hour or so will show no change. If now the electric current is started, in the course of ten minutes a pale band will begin to appear in the fin and extend from a little above the holes to the edge of the fin (Fig. 12). In twenty-

FIG. 12. Anterior dorsal fin of a dark smooth dogfish, *Mustelus*, which has been stimulated to the formation of light bands by a transverse cut near the anterior edge of the fin and by electric stimulation posterior to this cut. The resultant light areas are seen in the fin peripheral to the two regions of stimulation. Parker, *Biol. Bull.*, 1935, 68, 2, fig. 1.

five minutes this band may become as distinct as that produced by a cut. If in a given fin electric stimulation and stimulation by a cut are started at approximately the same time and in adjacent positions, two pale bands will form, one from each center of excitation, and in a

way so that they are indistinguishable one from the other. These results confirm the view held by Parker and Porter that the pale phase in *Mustelus* is due to the action on its melanophores of concentrating nerve-fibers which may be stimulated by an induction current as well as by being cut.

Combining the work of Lundstrom and Bard with that of Parker and Porter a reasonably clear picture of the color changes in *Mustelus* can be outlined. This fish darkens in consequence of a pituitary neurohumor

FIG. 13. Two newly born smooth dogfishes, *Mustelus*; lower one in the pale phase, upper one in the dark phase. Parker, *Biol. Bull.*, 1936, 70, pl. 1, fig. 2.

carried from the pituitary gland by the blood to the melanophores which are thereby induced to disperse their pigment. It blanches as a result of the action of concentrating nerve-fibers by methods that I shall discuss more fully later.

Mustelus canis is ovoviviparous, that is, its eggs are carried in the oviducts of the female till the young are very fully formed when they escape from the mother's body as active young fishes. A female may release from four to a dozen or more such young at a time, and these

at birth may measure from twenty-five to thirty centimeters in length. If the female dogfish is killed and the young within her are quickly removed by what may be termed a crude Caesarean operation, the pups, as they are called, if handled gently, remain momentarily passive even when immersed in sea water. After they have been for a fraction of a minute or so in their watery environment their gill movements begin and they will start swimming, but with somewhat unsteady equilib-

FIG. 14. Dermal melanophores of a newly born smooth dogfish, *Mustelus*, in the pale phase, pigment concentrated. Parker, *Biol. Bull.*, 1936, 70, pl. 1, fig. 3.

FIG. 15. Dermal melanophores of a newly born dogfish, *Mustelus*, in the dark phase, pigment dispersed. Parker, *Biol. Bull.*, 1936, 70, pl. 1, fig. 4.

rium. In a very short time, however, they are indistinguishable from pups normally born.

Fully formed young dogfishes when first removed from the female are darkish in tint, but they will respond immediately to the tone of the environment. In one instance three newly born pups were put at once into a black-walled illuminated tank. In twenty minutes they had darkened perceptibly and in a little less than an hour they were fully dark. Others that had been put directly after birth into a white-walled tank were

fully blanched in about an hour and ten minutes. The extreme degrees of tint in these young fishes (Fig. 13)

FIG. 16. Dorsal view of a pectoral fin from a newly born dogfish, *Mustelus*, showing two pale bands as the result of small transverse cuts. Parker, *Biol. Bull.*, 1936, 70, pl. 1, fig. 1.

were quite equal to those of the adults, and the pigment in their melanophores showed as much concentration (Fig. 14) and dispersion (Fig. 15) as did that in the mature fishes. When transverse cuts were made in the fins of these young fishes pale bands developed (Fig. 16) as in the adults. In all these respects the newly born pups of *Mustelus* agree with the adults,

and they may be said to come into the world with their chief systems of organs including their melanophores fully functional (Parker, 1936*b*).

III

THE KILLIFISH

THE killifish, *Fundulus heteroclitus*, is a small fish some three to four inches in length, found in the coastal and estuarial waters of the eastern shores of the United States from Maine to Texas. It can be transferred directly from fresh water to salt water and back again with impunity and in other respects as well it is very hardy. In consequence it is a favorite live-bait for winter fishermen and a very convenient animal for the experimentalist. Its skin is abundantly supplied with melanophores, the larger of which are often associated with iridocytes. It also possesses a large number of dermal xanthophores and a few local guanophores (Odiorne, 1933). Under appropriate circumstances dependent for the most part on the background, *Fundulus* may assume a pinkish, bluish, greenish, yellowish, or steel-black tone, or pass over into a pale gray or almost pearly white stage (Connolly, 1925). Some of these phases, such as the pinkish one, are reached only very slowly and may require days or even weeks for full accomplishment. Others, like the changes from pale to dark and the reverse, may be brought about in a few minutes. These latter changes, as might be expected, are dependent upon the action of the melanophores and have been studied much more generally than the others. The change from pale to dark and the reverse take place with remarkable certainty and rapidity (Fig. 17). When a pale *Fundulus* in a white-walled, illuminated vessel is transferred to one with black walls, the fish will change from its original pale tint to a dark one in a little less than a minute, though the full completion of this change

may require nearly two hours. When the reverse is tried and a dark fish is transferred from an illuminated black-walled vessel to a white-walled one the fish

FIG. 17. Dark phase (above) and pale phase (below) of the killifish, *Fundulus*, as the results of exposure to dark and to light backgrounds. Parker, *Four. Exp. Biol.*, 1934, 11, pl. 1, fig. 1.

blanches in a little over two minutes, though again the final stage may not be reached for some four hours. These changes take place in the times just given provided the temperature of the water in the experimental vessels is approximately 20°C . At lower temperatures the reaction times may be much lengthened, but under all circumstances darkening appears to be accomplished always more rapidly than blanching, a rule that holds for the corresponding changes of a number of other animals (Parker, Brown, and Odiorne, 1935). It is an interesting fact that when a normal *Fundulus*, either pale or dark in tint, is put in an environment of complete darkness it does not assume a dark tone as might be expected, but it blanches strikingly and remains pale (Parker and Lanchner, 1922). This fact, which has been already noted in other fishes by von Frisch (1911), makes it obvious that there must be a significant difference in these forms between being in the dark and seeing

black. Another feature that should be noted in passing is that a *Fundulus* from which the eyes have been removed is pale in darkness, as normal individuals are, but moderately dark in bright illumination. This darkening of blinded fishes in illuminated vessels occurs to an equal degree whether these vessels have black walls or white walls (Parker and Lanchner, 1922). Such environmental differences are responded to, as might be expected, only when the eyes of the fish are functional.

The general color conditions just described for *Fundulus* are exhibited by this fish the year round, but during the breeding season, the height of which is toward the end of June, all color responses are greatly intensified, especially in the males. In fact at this time of year the male is readily distinguished by a dark eye-like mark on its dorsal fin, a nuptial secondary sex-character, which is formed by an aggregation of melanophores in

FIG. 18. Four enlarged views of the dorsal fin of the killifish, *Fundulus*, showing the nuptial mark in the male (1, 2) and its absence in the female (3, 4). Figures 1 and 3 represent the dark phase and figures 2 and 4 the pale phase of this fish. Parker and Brower, *Biol. Bull.*, 1935, 68, 5.

the fin membrane (Fig. 18). This mark can be seen in the males from April to November, but not at other times of year (Parker and Brower, 1935). Such seasonal markings and the accentuated color changes that accompany them during the breeding season undoubtedly play an important rôle in the sporting of the sexes in mating. The less pronounced year-round changes of darkening and blanching are probably of a character purely protective.

Some of the observations recorded in the preceding paragraphs are not of great moment for the present discussion and have been little studied, but I have introduced them here that you may appreciate to some extent the complexity in the reactions of the melanophores in the killifish, and that you may have some understanding of what we are dealing with when we attack the problem of even the simpler color changes in this fish. The main features to be kept in mind in the following discussion are that in *Fundulus* under normal conditions the animal turns quickly dark in an illuminated, black environment, and less quickly pale in a similar white one. It is hardly necessary to say that the dark phase of the fish is one in which the pigment of its melanophores is fully dispersed, and the pale phase one in which this coloring matter is compactly concentrated.

We may now proceed to inquire into the mechanism of the melanophore changes in *Fundulus* and, since they are dependent upon the eyes, we are naturally led to ask first of all how much of a part nerves play in these reactions. A host of investigators have attempted to identify chromatophoral nerves by the very reasonable step of cutting given nerve-strands and then looking for possible color changes in the integumentary areas that have been thus denervated. This method was employed by Brücke, by Pouchet, by von Frisch, and many

others. It can be easily and conveniently used on the tail of *Fundulus*. The tail of this fish is a blunt, symmetrical organ like the free end of a spatula, supported by a system of over twenty fin-rays along which nerves pass from the root of the tail toward its free edge. A short cut made transverse to these rays and near their proximal ends severs one or two of them with their associated nerve-strands, thus producing an elongated denervated area extending from the cut to the free edge of the tail (Fig. 19). This cut, excepting in its immediate neighborhood, produces no disturbance in the blood supply to the denervated part of the tail, for collateral vessels are so numerous in this organ that a short distance along the band from the cut a normal flow of blood can always be seen under the microscope.

FIG. 19. Diagram of a caudal fin of a killifish, *Fundulus*, showing a band of dark melanophores produced by cutting radial nerves near the root of the tail. Parker, *Pro. Nat. Acad. Sci.*, 1934, 20, 307, fig. 1.

If a cut as described is made in a pale *Fundulus*, the denervated area will begin to appear as a darkened one within thirty seconds of the time of the operation, and the band will continue to deepen in tint till it reaches a maximum a few hours later. Such a band corresponds to the darkened areas produced by nerve cutting in the chameleons and other lizards (Brücke, 1852; Bert, 1875; Keller, 1895; Redfield, 1918; Hogben and Mirvish, 1928*a*, 1928*b*; Zoond and Eyre, 1934) as well as in a great variety of fishes (Pouchet, 1876; von Frisch, 1911; Wyman, 1924; Hewer, 1927; Giersberg, 1930; Fries, 1931; Smith, 1931*b*; Mills, 1932*a*; Parker, 1934*b*, 1935*c*).

As I have already stated, the majority of workers beginning with Brücke (1852) and extending down even to Sand (1935) have expressed the opinion that such severed chromatophoral nerves are paralyzed and that the associated melanophores after the severance of the nerve lapse into a state of inactivity comparable to the relaxed condition of inactive muscle.

FIG. 20. Diagram of a faded band in the tail of a killifish within which a new short cut has been made. This cut induced the formation of a new small band within the larger one showing that the severed nerve-fibers of the original band were still active. Parker, *Pro. Nat. Acad. Sci.*, 1934, 20, 308, fig. 3.

That this view of the relation of the chromatophoral nerves and their associated melanophores is probably erroneous is seen from the following experiments (Parker, 1934a). If a dark caudal band is induced in a pale *Fundulus* in the way already described and the fish is kept in a white illuminated vessel, the band will gradually fade, as was first pointed out by Pouchet in 1876 and subsequently confirmed by von Frisch (1911). In the caudal band of *Fundulus* this fading will occur in a few days more or less variable with different individuals. If, after the caudal band in *Fundulus* has faded, a new

transverse cut is made within the area of the old band and slightly distal to the original cut, a wholly new dark band will appear reaching from the new cut over a part of the old band to the edge of the tail (Fig. 20). The formation of this new band could not possibly take place if the nerve-fibers originally cut had been paralyzed by that step. The formation of the second band within the limits of the first is to be interpreted, in my opinion,

as a temporary reactivation of the original fibers with a corresponding response of the melanophores. When these fibers were originally cut they were not paralyzed by this operation, but were profoundly stimulated, and this stimulation continued, though with diminishing energy, for one or more days as indicated by the state of their effector organs, the melanophores. This at least is the interpretation that I have been led to put on this phenomenon. I am fully aware that it is quite contrary to the teachings of conventional neurophysiology. But this type of physiology has been developed on the basis of the nerve-muscle preparation, and there may be important differences between such preparations and those based on melanophores.

If cut melanophoral nerve-fibers remain active for a long time after severance, there must be a continuous flow of activating impulses from the region of the cut to the melanophores that constitute the dark band.

One test of the correctness of this view would be to attempt to block such a flow. This cannot well be done with drugs because of the readiness with which they diffuse, but it can be accomplished by the application of cold. If a sharply bent, capillary glass-tube carrying a chilled mixture of water and alcohol several degrees below zero centigrade is applied to a region on a fully formed caudal band about midway its length, a very

FIG. 21. Diagram of a fully formed band in the tail of a killifish to which a cold block (*A*) had been applied with the result that the distal half of the band faded in about a quarter to half an hour after the application of the block. Parker, *Pro. Nat. Acad. Sci.*, 1934, 20, 309, fig. 5.

remarkable occurrence will be noticed. The band proximal to the region of the application of the cold tube will remain unchanged, but that distal to it will in the course of half an hour or less gradually fade out (Fig. 21). This is what would be expected if the band was

maintained by a continuous flow of impulses from the cut toward the free edge of the tail.

FIG. 22. Diagram of a caudal fin of a killifish across a part of which a capillary tube (A) carrying a chilling mixture was placed. The cut (B) was followed by the formation of a dark band which reached from the cut to the chilled area but did not enter it. The cut (C) gave rise to a dark band which reached to the edge of the tail. Parker, *Pro. Nat. Acad. Sci.*, 1934, 20, 307, fig. 2.

A cold block may be used in still another way to test this question. If the cold tube is applied to a spot near the center of the tail of *Fundulus* and a denervating cut is made some distance proximal to the region of application, a dark band will begin to form and will extend from the cut to the region of the cold block but, as might be expected, will not pass beyond this block. If now a cut is made immediately distal to the block and in line with the first one, an additional band will form from the new cut to the edge of the fin (Fig. 22). From this experiment two

important conclusions may be drawn: first, that cold in the neighborhood of 0°C . serves as a real block to nervous impulses over chromatophoral fibers; and, second, that what is transmitted from the central organs over these fibers is not an inhibitory influence that is checked when the fibers are cut, but a true activating influence that may be excited locally by severing the nerve.

Precisely what induces the excitation at the cut and maintains it cannot now be stated definitely. Since the tail of the fish is in almost continuous lateral movement during the life of the animal it might be supposed that after the cut is made the continual rubbing of the severed ends of the nerve-fibers in the wound is the occasion of the prolonged activity of these fibers. That such, however, is not the case can be shown by the simple experiment of cutting a window in the tail instead of making merely a transverse slit (Fig. 23). Under such circumstances the nerve-fibers are no longer rubbed on the rough surfaces of the wound, and yet from a window a caudal band is formed and maintained precisely as it is from a simple transverse cut. Mechanical stimulation probably has nothing to do with the maintenance of the band. There are, however, enough other disturbances in the cut to account for continuous stimulation, but precisely what these disturbances are is at present unknown.

Nerves that are cut as these caudal nerves have been would naturally be expected to undergo degeneration, and it is reasonable to ask whether this process plays any part in the activities under consideration. After a denervated darkened band in the tail of a *Fundulus* has blanched, the activity of its severed nerve-fibers can be tested by recutting them. If the band darkens a second time, the fibers must be regarded as still functionally active; if not, they may reasonably be suspected of de-

FIG. 23. Diagram of a caudal fin of a killifish in which a small window was cut resulting in the formation of a dark band. Parker, *Pro. Nat. Acad. Sci.*, 1934, 20, 308, fig. 4.

FIG. 24. Tail of a killifish, *Fundulus*, eighteen days after a primary cut and immediately after a more proximal secondary cut had been made resulting in the formation of a dark block between the two cuts. There is no evidence of the regeneration of dispersing nerve-fibers. Parker and Porter, *Jour. Exp. Zool.*, 1933, 66, pl. 1, fig. 3.

of dark melanophores will appear between the two cuts showing that the central ends of the chromatophoral nerves are still normally active (Fig. 24). This darkening, however, will not extend as a rule distal to the first cut, a condition which shows that at this stage no new nerve-fibers have grown out from the old nerve-stump into the denervated area. At about the eighteenth day after

generation. By this means it can be shown that the dispersing chromatophoral nerve-fibers of *Fundulus* remain active for some four to five days after they have been cut. Functional inactivity begins to appear in about five days, thereby showing that the early stages of degeneration have set in. If at any time up to the eighteenth day after the initial cut was made, a second cut is made slightly proximal to the first one, a block

FIG. 25. Tail of a killifish, eighteen days after a more proximal secondary cut had been made from which a dark band extends partly over the old primary band and thus shows evidence of some regeneration in the dispersing nerve-fibers. Parker and Porter, *Jour. Exp. Zool.*, 1933, 66, pl. 1, fig. 4.

the initial cutting or shortly thereafter, a second cut in preparations of the kind described will be followed by short dark streaks that can be traced into the region of denervation (Fig. 25). As can be seen in fishes prepared for this purpose and experimentally tested from day to day, these dark bands extend farther and farther each day till eventually they reach the edge of the fin (Fig. 26). They are of course due to the presence of melanophores with dispersed pigment and reflect the progressive regeneration of the dispersing nerve-fibers. In *Fundulus*, under the circumstances described, this regeneration begins about the eighteenth day after the initial cut and is completed on about the twenty-fifth day. The approximate distance covered by the growing nerve in these seven days is some six millimeters, and the rate of regenerative growth consequently is about 0.8 millimeter per day (Parker and Porter, 1933).

FIG. 26. Tail of a killifish twenty-five days after a primary cut had been made. The proximal secondary cut induced the formation of a complete new caudal band showing that the regeneration of the dispersing nerve-fibers had been fully established. Parker and Porter, *Jour. Exp. Zool.*, 1933, 66, pl. 1, fig. 6.

By an ingenious method that is an improvement over the one just described, Abramowitz (1935) has made a second determination of the rate of regeneration not only for the dispersing fibers but also for the concentrating ones in *Fundulus*, for there is evidence, as we shall see later, for two kinds of chromatophoral nerve-fibers in this fish. In the method employed by Abramo-

witz a *Fundulus* with a blanched caudal band, on which the rate of regeneration in the dispersing nerve-fibers is to be measured, is kept in a white-walled vessel. When a measurement is to be made the fish is placed for five minutes or more in a black-walled receptacle. Here it naturally darkens, including such part of its caudal band as has regenerated its dispersing fibers. The extent of this part is measured and the fish returned to the white-walled vessel where it remains till another measurement is desired. The same method can be applied to the concentrating nerve-fibers except that in this instance the experimental fish is retained in a black-walled receptacle and transferred temporarily for testing to a white-walled one. In this instance the extent of the pale band beyond the initial cut is of course what is measured. By these methods Abramowitz calculated the rate of regeneration in both sets of nerve-fibers and found them in both instances to be about 0.5 millimeter a day. The earlier determination by Parker and Porter, 0.8 millimeter a day, is not far from this more accurate figure by Abramowitz, and both fall within the range of the rates already published for the regeneration of nerves in the frog (1.34 mm. per day, Harrison, 1910; 0.24 mm. per day, Williams, 1930; 1.44 to 0.20 mm. per day, Speidel, 1933). As can be seen from these studies, the degeneration of the chromatophoral nerves in *Fundulus* begins some five days after they have been cut, and regeneration is a matter of weeks later. Obviously these degenerative and regenerative processes have nothing to do with the formation of the caudal bands and their blanching, all of which may occur in a day or so after the formation of the band. Over the early period of four or five days the chromatophoral nerves are functionally active, and this activity quite reverses the older conception concerning severed nerves. After they are

cut they are not at once paralyzed, as was formerly thought, but they are by the very act of cutting thrown into a state of superactivity which may last in *Fundulus* for two, three, or more days. The resulting dispersion of pigment in the associated melanophores then is not indicative of a stage of inactivity comparable with that of relaxed muscle, but is rather one of unusual excitation. From these several lines of inquiry it appears to be reasonably well established that the melanophores of *Fundulus* are provided with nerve-fibers whose action incites a dispersion of pigment in these color-cells. The dark phase in this fish results then from the stimulation of its melanophores by nerves, and the fibers concerned may be designated, in consequence of this action, as dispersing fibers. It must be evident at once that this opinion is quite contrary to that which for the last half-century has been generally espoused by the majority of workers in this field. Nevertheless the facts presented in the preceding pages warrant, in my opinion, the acceptance of this newer conception.

If from the standpoint of the most recent work the dark phase of *Fundulus* is due to the action on its melanophores of a system of dispersing nerve-fibers, what can be said about the pale phase? In the color changes of fishes and of reptiles the pale phase has long been regarded as occasioned by the direct action of chromatophoral nerves. This view has been held in consequence of the ease with which blanching can be excited through electrical stimulation of integumentary and other nerves. Such responses were observed as early as 1852 in the chameleon by Brücke, whose observations on this point have been confirmed and extended on the same animal by Hogben and Mirvish (1928a, 1928b) and on *Phrynosoma* by Redfield (1918). The same appears to hold for fishes, as was demonstrated on

Phoxinus by von Frisch (1911) and on numerous other fishes by Spaeth (1913), Schaefer (1921), Wyman (1924), and others. As Sand declares (1935), electrical stimulation of nerves in fishes has always been found to cause the melanophores to contract.

This generalization is certainly supported by what is known of *Fundulus*. If one or two rays in the tail of a dark *Fundulus* are included between the electrodes of an inductorium, on making the currents a pale band develops between the region stimulated and the edge of the tail. If, following the procedure of von Frisch, the electrodes are applied to the occipital region of *Fundulus* so as to stimulate its medulla, the whole fish becomes pale with almost incredible quickness. These observations, which have been many times repeated and confirmed, point indubitably to the conclusion that the chromatophoral nerves of *Fundulus* contain fibers that are concerned with the concentration of pigment and therefore with the consequent blanching of the fish. These fibers may, therefore, be designated concentrating fibers in contrast with the dispersing fibers already discussed. In *Fundulus* then it would appear that its melanophores are provided with a double equipment of nerve fibers, one set to excite dispersion and the other concentration of pigment.

The conclusion thus arrived at raises the much-disputed question concerning the double innervation of effectors. So far as chromatophores are concerned, this idea was apparently first suggested in 1875 by the French physiologist Paul Bert, who in a very incidental way and without proof of any serious kind advanced it in explanation of ordinary chromatophoral responses. Bert's declaration never received a thoroughgoing consideration and has been allowed to drift on in a rather indeterminate way. The number of investigators who

believe that their researches on the whole are favorable to Bert's view (Carnot, 1896; Sollaud, 1908; Redfield, 1918; Kahn, 1922; Giersberg, 1930; Smith, 1931*a*) are about equal to those who take the opposite stand (Spaeth and Barbour, 1917; Hogben, 1924; Gilson, 1926; Sand, 1935). The inconclusiveness of much of the work on this question probably resulted from the fact that it involved experiments with drugs mainly from the standpoint of mammalian physiology, a somewhat uncertain procedure when transferred to the lower vertebrates, and further that no small part of it was done on amphibians where as we now know, thanks principally to Hogben and his associates, chromatophoral nerves are mostly, if not entirely, absent.

It is not my purpose here to attempt to deal with this question in a general way, for, as will be shown toward the end of this essay, the truth is that double innervation is not a general question. Melanophores may have double innervation, as I believe to be the case in *Fundulus*, or single innervation as has been shown to be true of the dogfish, or no innervation at all as appears to be the case in lampreys (Young, 1935) and in the frog. The point that I wish to discuss here is whether there is reason to assume double innervation for the melanophores in *Fundulus*.

Some of the evidence leading up to this view has already been given, but there remain certain important aspects of this question still to be considered. In 1932 Mills pointed out that a close scrutiny of the melanophores at the edge of a caudal band in *Fundulus* showed very important differences depending upon the state of the color-cells. The differences here considered are best seen when the band itself is somewhat blanched. If under such circumstances a given fish is darkened by being kept in an illuminated black-walled vessel, the

pigment in certain melanophores at the edge of its band will be found to be as fully dispersed as those on the tail in general. When this fish is made to blanch by a short retention in an illuminated white-walled vessel these same melanophores will be found not to have concentrated their pigment fully but to have come to rest with their coloring matter in a position between the extremes of concentration and of dispersion; in other words, these melanophores can disperse their pigment fully but cannot concentrate it fully. In a corresponding way other melanophores can be found also at the edge of the band that can concentrate their pigment fully but cannot disperse it fully. These conditions, so far as the band as a whole is concerned, may cause its edge to appear to shift slightly, depending upon whether the tail as a whole is dark or pale. An understanding of this peculiar situation is possible on the assumption of double innervation, but not on that of single innervation. When nerve-strands are cut, as in the excitation of a caudal band, both kinds of fibers, assuming both kinds to be present, must of course be severed. Nerve-fibers in the tail do not pass out into that structure on strictly radial lines, but scatter somewhat irregularly. Consequently near the edge of a caudal band it would not be surprising to find certain melanophores whose concentrating fibers had been eliminated by the cut, but whose dispersing fibers were still intact, and others in which the reverse was true. Under these circumstances some melanophores would be open to excitation for dispersion but not for concentration and vice versa, a condition of affairs that would result in exactly what has been observed. Double innervation then will explain this peculiar state; single innervation will not.

Another aspect of the problem of double innervation is found in the regeneration of chromatophoral nerves.

This is pointed out by Abramowitz in work which is in process of publication and from which I am permitted to make the following excerpt. When new nerve-fibers grow out from a proximal stump of a severed caudal nerve they take, as already mentioned, a distal course over the previously formed band and grow at an approximate rate of half a millimeter a day. If during this regeneration the progressing front of the growing nerve as indicated by the melanophores under its control is studied closely, it will often be found to vary in position, depending upon the states of the melanophores used in the test. If the fish is rendered dark by keeping it in a black-walled vessel, the front of the regenerating band of nerve as judged by the melanophores may be at one place; if the fish is immediately thereafter blanched by being put into a white-walled vessel the front as judged in the same way may be measurably elsewhere. In certain fishes the developing front as shown by dispersed melanophores may be in advance of that shown by the concentrated ones; in other fishes the reverse may be true. This disagreement in the position of the advancing front as shown by the two states of the melanophores, a disagreement that in a way is like that already described on the edge of the fully formed band, is quite consistent with the idea of double innervation, but very difficult if not impossible to reconcile with single innervation. Obviously the two sets of regenerative nerve-fibers do not always grow forward at precisely the same rate. In some instances the concentrating fibers are in advance, in others the dispersing fibers. Here again the idea of double innervation is a necessary part in the explanation of a well-ascertained functional state, that concerned with the regeneration of melanophore nerves.

In consequence of these two important lines of evidence, one from the work of Mills and the other from

that of Abramowitz, as well as from the original consideration already set forth, it seems fair to conclude that the melanophores of *Fundulus* possess a double innervation, and that the two sets of nerve-fibers, dispersing and concentrating, are real elements in the neuro-melanophore organization of this fish. It is interesting to observe that in the figures of melanophore nerves in fishes published in 1893 by Ballowitz (Fig. 27),

FIG. 27. Innervation of a chromatophore from the perch. Ballowitz, *Zeit. wiss. Zool.*, 1893, 56, pl. 38, fig. 21.

each color-cell receives several nerve-fibers and not simply one, as muscle-cells ordinarily do. Of these nerve-fibers, which are sometimes rather numerous, one at least is presumably a concentrating fiber and another a dispersing one. From this standpoint it is indeed possible that melanophores may differ somewhat in their functional capacities depending upon a larger or a smaller number of one or other kind of fiber. Thus some cells may be more active in concentrating their pigment than in dispersing it in consequence of a pre-

dominant concentrating innervation. But this point, so far as I am aware, is purely speculative.

Evidence for double innervation such as that which has been presented is not easily and quickly gathered. The only other fish that has been exhaustively studied from this standpoint is the common freshwater catfish, *Ameiurus nebulosus*, whose melanophore system (Parker, 1934*d*) appears also to involve two sets of nerves.

Perhaps the most important advance made in the physiology of chromatophores during the last decade and a half has been the establishment of the fact that pituitary secretions are often of the utmost significance in color changes. Now what part do these secretions play in the melanophore activities of *Fundulus*? To test this question Matthews (1933) removed the pituitary glands from a number of killifishes, and after their recovery he subjected them to changes of environment to ascertain whether they had lost to any extent their capacity to alter their tint. Having observed no such loss Matthews concluded that this gland in *Fundulus* was of little or no importance in controlling color changes. Matthews, however, made the interesting observation that an extract from the pituitary gland of this fish when applied to an isolated scale was followed by a concentration of pigment in the melanophores of the scale. Rather the reverse of this effect was recorded by Kleinholz (1935) who showed that when pituitary extract from a *Fundulus* was injected into another on whose tail was a partly blanched caudal band, this band darkened though the fish as a whole did not. These various observations demonstrate that under normal conditions the pituitary gland in *Fundulus* is probably of no importance in the control of the color changes, though the exceptional responses obtained from its secretion as applied to isolated scales by Matthews and to caudal bands by Kleinholz call for further elucidation.

IV

NEUROHUMORS

THE preceding extended examination of the melanophore system in *Fundulus* leads to the conclusion that the pituitary secretions in this fish in all probability play no real part in its color changes, which seem to be controlled exclusively by two sets of autonomic nerves, one concentrating and the other dispersing in function. It might seem that this would be the end of our quest, but there are other phases of the subject that lead us on to rather novel and interesting fields of inquiry. These have to do with the way in which the nerves control the melanophores, a process which is approachable in the blanching of caudal bands in fishes.

Dark spots produced on the skins of fishes by cutting integumentary nerves were long ago recognized as temporary. Pouchet in 1876 noted that these darkened regions subsequently became as pale as the rest of the fish, and von Frisch (1911) observed that the contrast between the dark area and the pale general surface of a minnow may vanish in from twelve to fourteen days. Smith (1931*b*), who worked also on the minnow, confirmed these early observations. When the ophthalmic branch of the trigeminal nerve on one side of a minnow's head is cut, a dark area appears and covers the anterior dorsal aspect of the half of the head concerned. This area disappears in the course of several days when the fish is kept on a white background, but it does not disappear when the fish remains on a black background. This led Smith to the conclusion that some non-nervous agency was here involved. This phenomenon was investigated in *Fundulus* by Mills (1932*b*) who showed

that a caudal band in *Fundulus* did not disappear uniformly as a whole but was subject to a gradual reduction which beginning on the two lateral edges of the band spread slowly toward its axis, the last portion of the band to be seen. The steps in the peripheral disappearance of the bands in *Fundulus* was demonstrated photographically by Parker (1935c) who succeeded with the help of Abramowitz in photographing from hour to hour identically the same area in a fading caudal band on this fish. At the outset all melanophores across the whole dark band had their pigment about equally dispersed (Fig. 28). Some nine hours later those on the

FIG. 28. Caudal band of a living killifish a quarter of an hour after it has been formed by the cutting of a single fin-ray. This ray is represented at the region of the photograph by its four branches. The pigment in all denervated melanophores is fully dispersed. Parker, *Proc. Amer. Philos. Soc.*, 1935, 75, pl. 3, fig. 13.

edge of the band showed greater pigment concentration than those near its axis (Fig. 29). Finally after about two and a quarter days those near the axis had as con-

FIG. 29. The same portion of the caudal band as is shown in Fig. 28 photographed nine and a quarter hours after the initiating cut had been made. The pigment in the marginal denervated melanophores is more concentrated than that of the axial color-cells. Parker, *Pro. Amer. Philos. Soc.*, 1935, 75, pl. 3, fig. 16.

FIG. 30. The same portion of the caudal band as is shown in Fig. 28 photographed fifty-four hours after the initiating cut had been made. The pigment in all denervated melanophores is very fully concentrated. Parker, *Pro. Amer. Philos. Soc.*, 1935, 75, pl. 3, fig. 18.

concentrated pigment as those on the edge, and the band as a whole had become quite pale (Fig. 30). In other words, the concentration of pigment begins on the edge of the band and, as was clearly stated by Mills, proceeds toward its axis. Subsequently Abramowitz found that relatively large dark areas, such as may be produced on one side of the head of *Fundulus* by cutting the ophthalmic nerve of that side, also disappeared by peripheral reduction.

This method of blanching is of no small significance for the topic at hand. It indicates that the influence that induces blanching proceeds not from below, in which case the whole dark area would become pale at the same time, but that it invades the area from the side. The importance of this will be more fully appreciated if we turn first to the structure of the tail.

The tail of *Fundulus*, like that of most bony fishes, is made up of two layers of skin supported within by relatively stout fin-rays separated by considerable intervals (Fig. 31). A few melanophores are lodged in the

FIG. 31. Cross section of the caudal fin of the catfish, *Ameiurus*, showing near the middle a fin-ray cut across and on each side above and below the integument with melanophores. Parker, *Four. Exp. Zool.*, 1934, 69, pl. 3, fig. 14.

cavities of the fin-rays, but the great majority of them rest on the deep surface of each layer of skin (Fig. 32). The space between the two layers of skin where it is not occupied by the fin-rays is filled with loose connective

tissue, and through this connective tissue and the fin-rays run the blood-vessels and nerves that supply the tail. In this way the two layers of melanophores in the tail are subtended by tissue rich in blood and lymph. Yet when a dark caudal band fades, as has just been pointed out, it does so not uniformly as though it were

FIG. 32. Section of the integument of the caudal fin of the catfish, *Ameiurus*, showing two small spherical melanophores in the epidermis and three large ones in the derma. Parker, *Four. Exp. Zool.*, 1934, 69, pl. 3, fig. 15.

acted on from below by some constituent of the adjacent blood and lymph, but laterally as though it were attacked at its edges. It can, however, be made to fade uniformly by injecting into the circulation of a given fish a small amount of dilute adrenalin, after which the whole band will blanch evenly, the axis as rapidly as the edges.

Further evidence that the blood of a *Fundulus* does not aid in blanching a band is seen in the fact that if the defibrinated blood from pale fishes is injected into a dark one no change in tint is seen in the recipient. And the reverse is also true, namely, blood from a dark fish has no effect upon the tint of a pale one (Matthews, 1933). The fading of a caudal band as ordinarily ob-

served in *Fundulus*, therefore, is not to be attributed to some action of the subjacent blood and lymph. It must be due to some influence that enters the band from the side.

It is a noteworthy fact, as Smith (1931*b*) pointed out, that bands or like areas do not fade when fishes are kept permanently on a black background. Fading occurs only when the adjacent region in the tail or other part of the body is pale, as though some agent from the neighboring pale area made its way into the band. Such an agent might well be a neurohumor produced in the pale area by the concentrating nerve terminals of that region and transmitted laterally into the band.

Such a neurohumor may be conceived of as spreading slowly from the region of its production into that of the band and of inducing there the same kind of change that it is capable of accomplishing over the rest of the body. That the action is in all probability a slow diffusion of this kind is not only shown in the beginning of these changes on the periphery of the band or other dark areas, but by the fact that bands of different widths take different amounts of time in which to disappear (Parker, 1934*b*). A band of a width of one millimeter on a pale *Fundulus* will require on the average a little over twenty-six hours in which to disappear; another two millimeters in width calls for nearly fifty-two hours in which to blanch. These determinations support the hypothesis that a neurohumor produced in the pale region of the tail and responsible for the tint of that region diffuses from the pale region into the band and thus causes it in time to blanch.

Not only is there reason for believing that concentrating fibers act on melanophores through concentrating neurohumors, but also that dispersing fibers act on melanophores by corresponding means. After a caudal

band on a pale *Fundulus* has become about as pale as the rest of the fish, the animal may be placed on a black background, whereupon it will darken after a few minutes in all parts except the band. This will remain pale for some time, but will in the course of several hours gradually darken till finally it is as dark as the rest of the fish. Such procedure may be repeated back and

forth many times, the fish blanching or darkening quickly, and the band following the general tint of the fish, but always with a lag of some hours. A situation of this kind is clearly explicable on the assumption of two sets of neurohumors, one dispersing, the other concentrating, and each produced by its appropriate nerve endings.

FIG. 33. Diagram of the caudal fin of a hypophysectomized catfish, *Ameiurus*, in the dark phase. Above is a faded caudal band and below a dark newly excited one. Parker, *Jour. Exp. Zool.*, 1934, 69, pl. 2, fig. 6.

Evidence for the lateral spread of the dispersing neurohumor can be seen in certain experiments that are better carried out on the tail of the catfish, *Ameiurus*, than on that of *Fundulus*.

The tail of the catfish presents much the same conditions as that of *Fundulus*. If one of its fin-rays is cut, a dark caudal band results much as in the killifish. This band will likewise blanch (Fig. 33) if the catfish is kept in a white environment, though the process is slower than it is in *Fundulus*. If now in a catfish with a pale caudal band two new dark bands are made by cutting the fin-rays adjacent to that of the pale band, and the cuts for the new bands are made not near the root of the tail as that for the pale

band was, but about halfway out on the rays toward the tip of the tail, the result will be a central pale band whose distal half will be abutted laterally by dark bands. The proximal half of the pale band will be surrounded only by the pale portions of the tail. Under such conditions the distal half of the pale central band which is flanked by the dark half-bands will be seen to darken slowly, whereas the proximal half will remain light (Fig. 34). This experiment shows quite clearly how adjacent dark areas may bring about a dispersion of pigment in a pale area, a change easily understood from the standpoint of an invading dispersing neurohumor. The darkening just described occurs only when the invaded region of the pale band is a denervated one. If the dark half-bands are excited on either side of an innervated pale ray, no such deepening of tint occurs (Fig. 35). Apparently the normal concentrating fibers of such an innervated area are too active in maintaining the pale state to be overcome by an invading dispersing neurohumor (Parker, 1934^d).

FIG. 34. Diagram of the caudal fin of a hypophysectomized catfish with a faded caudal band between two newly cut dark half-bands. The half of the faded band not flanked by the new dark bands has remained pale; the half flanked by the dark bands has darkened slightly. Parker, *Jour. Exp. Zool.*, 1934, 69, pl. 1, fig. 5.

According to this general view, then, the two sets of melanophore nerves in *Fundulus* act on its color-cells through appropriate neurohumors which are produced by the proper nerve terminals and which excite in one

case pigment concentration and in the other pigment dispersion (Mills, 1932*b*).

It is no easy task to ascertain the nature of these neurohumoral substances in *Fundulus*. Attempts to extract them from the skin of this fish have failed, probably because they are so much involved in the scales that it is impossible to bring solvents easily into close contact with them. This, how-

FIG. 35. Diagram of the caudal fin of a catfish with a normal innervated band between two newly cut dark half-bands. The intermediate band retains its pale tint throughout its whole length. Parker, *Jour. Exp. Zool.*, 1934, **69**, pl. 2, fig. 7.

ever, is not true of all fishes. In some, as will be shown presently, they have probably been in a measure isolated. One point concerning their nature in *Fundulus* seems to be clear, namely, that they are not carried in the blood. As already stated, the defibrinated blood of a pale *Fundulus* has no effect on the tint of a dark fish and vice versa. This indicates that these particular neurohumors are not soluble in water. If they are not open to aqueous solution the only other probable means of dissolving them is oil or fat, and it is my opinion that these neuro-

humors are oil-soluble (Parker, 1933*a*, 1933*b*) and are transmitted, not through the lymph or other watery fluids between cells, but from cell to cell by means of their lipoid or oily constituents. Under such circumstances transmission would be slow, as in fact it is known to be, and would be limited to tissues in which the cells are more or less in contact with one another. In this respect the melanophores of *Fundulus* are a favorable

group of cells, for their processes are so richly branched and intertwined that the necessary contacts for such a transmission must be more than abundant.

The conception to which we are finally led respecting the control of melanophores in *Fundulus* is as follows: this control is accomplished through two sets of autonomic nerves, concentrating and dispersing, and, though it is what would be termed a strictly nervous control, it is nevertheless based upon a special type of hormone, a neurohumor, which ordinarily passes directly from the nerve terminal to the effector cell, the melanophore, over an almost submicroscopic distance, but under other circumstances may make its way over stretches of a millimeter or two from its region of origin to distant effectors by way of the lipoid constituents of the intervening tissues.

As a fish from which to attempt the extraction of an oil-soluble neurohumor, *Mustelus* is much more favorable than *Fundulus*. The phase of *Mustelus* that is suspected of being associated with such a neurohumor is the pale one, and the parts that show this phase to best advantage are the fins. Dogfishes were therefore put in a white-walled illuminated tank and after a few days, when they had become fully blanched, they were killed and their fins removed. It was a matter of good fortune that in the preparation of the fins the cutting of nerves intensified their paleness rather than the reverse. The fins immediately after their removal were ground to a pulp in an ordinary kitchen grinder, and the pulp from the fins of one ordinary dogfish was then thoroughly mixed with about two cubic centimeters of Italian olive oil. This mixture was further ground by hand for about half an hour in a rough porcelain mortar till it reached the consistency of a thick paste and then it was set aside to undergo extraction. In most in-

stances it was sterilized by heat before it was extracted, but in the beginning this step was avoided. Whether the paste was sterilized or not, its extraction was always carried on at the low temperatures of an ordinary ice refrigerator. After the paste had stood some fifteen hours or so, it was mixed with its own volume of sterile seawater, and the thick liquid that resulted was set aside to allow the oil to rise to the top. In this way there was collected a water-and-oil emulsion which after having been roughly filtered through sterile cheesecloth was vigorously agitated and injected subcutaneously in appropriate amount into a dark dogfish. Very soon after the injection had been made there commonly appeared on the skin of the dogfish and a little in front of the point of insertion a few small white spots which however soon disappeared. As these spots appeared when small amounts of indifferent fluids were injected as checks they were regarded as of purely operative origin. In from one to two days after the injection relatively large pale areas made their appearance in the skin immediately over the region into which the fin extract had been introduced (Fig. 36). These large areas were very persistent and, as could be shown under a low power of the microscope, they were produced by the concentration of melanophore pigment. That the pale skin included in these spots was essentially normal was demonstrated by the injection of pituitrin into a fish with such a spot. Shortly after an injection of this reagent had been made, particularly if the region of injection was close to the pale spot, it disappeared by the darkening of its melanophores only to return after a few hours as the effect of the pituitrin wore off.

These large pale spots were not produced by injections of seawater, oil, oil extracts of dark fins or of muscle, seawater extracts of pale fins, or defibrinated

FIG. 36. Left side of the trunk of a smooth dogfish, *Mustelus*, in the region of the anterior dorsal fin showing a secondary light spot due to an injection of 0.5 cc. of an emulsion of olive-oil extract of blanched fins and seawater made a little over a day previously. Parker, *Jour. Gen. Physiol.*, 1935, 18, 840, fig. 1 A.

FIG. 37. Right side of the same fish as is illustrated in Fig. 36 showing no change of color after the injection of 0.5 cc. of an emulsion of olive-oil and seawater. Parker, *Jour. Gen. Physiol.*, 1935, 18, 840, fig. 1 B.

blood from pale or from dark fishes (Fig. 37). They were produced from oil extracts, sterilized or not sterilized, of pale fins, and from cold ether extract and Soxhlet ether extracts from the same. These various tests lead to the conclusion that the induced pale areas in *Mustelus* are due to the action of some substance that can be extracted from the pale fins of this fish by olive oil or ether. The exact source of this substance cannot be stated, for it has been taken from the whole pale fin only. That it is not in dark fins and not soluble in water leads to the conclusion that it is in all probability the concentrating neurohumor concerned with the nervous blanching of *Mustelus*, but proof of this view is far from complete. The few known properties of the substance are its solubility in olive oil and in ether, its insolubility in water, and its resistance to dry heat up to 110° C. It is probable that even in the oily Soxhlet extracts it was present at most in extremely small amounts (Parker, 1935b).

The only other fish that has been examined for the possible presence of oil-soluble neurohumors is the catfish *Ameiurus*. In this fish dark and pale phases are well marked (Fig. 38) but the dark phase is the only one favorable for study. *Ameiurus* (Parker, 1934d) has a melanophore system almost a duplicate of that of *Fundulus* except that in addition to concentrating and dispersing fibers *Ameiurus* has an active pituitary neurohumor which supplements the function of its dispersing nerves. Extracts of the skins and fins of dark catfishes were prepared as in the case of pale dogfishes, and the final extract was injected subcutaneously into light catfishes. This operation was followed in a little less than an hour by the formation of dark splotches on these fishes (Fig. 39). Such splotches, which were caused by the dispersion of pigment in the melanophores of the

FIG. 38. Pale phase (above) and dark phase (below) of the common catfish, *Ameiurus*. Parker, *Jour. Exp. Zool.*, 1934, **69**, pl. 1, fig. 1.

FIG. 39. A catfish, *Ameiurus*, into which an injection of olive-oil extract of the dark fins and skins of five other catfishes had been made anteriorly from the black dot below the adipose fin. The resulting dark area is superficial to the region where the injected fluid escaped from the needle point. Parker, *Jour. Exp. Biol.*, 1935, **12**, pl. 1, fig. 2.

region concerned, disappeared spontaneously after a few days. When they were first formed they could be temporarily obliterated by an injection of adrenalin. Extraction of the skin of *Ameiurus* by ether, hot or cold, yielded residues that were slightly active in darkening the skin, but they were by no means so effective as were the ether extracts in the case of *Mustelus*. However, the evidence from the catfish supports the view that in *Ameiurus* a dispersing neurohumor is present which is soluble in oil and in this respect resembles the concentrating neurohumor of *Mustelus*.

The survey that has just been made of the means by which the melanophores of *Fundulus*, of *Mustelus*, and of other related species of fishes are activated indicates with reasonable certainty that the distinction between excitation by nerves and excitation by hormones is not a fundamental one. In what is ordinarily called direct stimulation of melanophores by nerves, as occurs for instance in *Fundulus*, there is sufficient ground to assume that of the two sets of nerve-fibers present each one when active produces a substance, a neurohumor, that can excite in a melanophore an appropriate response. This neurohumor is believed to be produced by the numerous nerve-terminals that surround the color-cell. It must pass from its region of origin, the terminal organ, over the almost submicroscopic space to the responding cell. In the sense that it passes from one place to another it is a hormone, but it is a hormone that ordinarily travels over only a very short distance. However, as already demonstrated in *Fundulus*, it may pass over as much as a millimeter or so of intervening space. It is therefore in all essential respects as much a hormone for the activation of melanophores, as the pituitary secretion is. So far as transmission is con-

cerned the pituitary secretion differs from that in the fin only in the much greater distance that the former must cover (Parker, 1934*b*). As previously suggested, all these agents, be they short-range or long-range, activate the melanophores in essentially the same way. Hormonal excitation and nervous excitation so far as color-cells are concerned are really one in principle; both are carried on by special hormones, the activating neurohumors.

It would be quite impossible at present to attempt a catalogue of neurohumors. From what has been mentioned in discussing the conditions in *Fundulus* and in *Mustelus* there appear to be at least two classes of these substances, the water-soluble and the oil-soluble or, as they have been designated, hydrohumors and lipohumors (Parker, 1935*b*). Hydrohumors are soluble in water and especially in blood, lymph, or other watery body fluids. In consequence they spread rapidly and far and ordinarily bring about responses over the whole animal. They are well represented by the chromatophoral secretions of the pituitary gland as seen in *Mustelus*, *Ameiurus* and a host of other creatures. Lipohumors are soluble in lipoids, fats, fat solvents and the like. Since such substances are essentially stationary in the animal body, the lipohumors after dissolving in them must diffuse through them and consequently move very slowly from place to place. Lipohumors are therefore relatively local in their action and do not excite responses of the body as a whole. They are appropriate to animals that can change their color patterns and maintain them thus changed as, for instance, certain flatfishes. When such fishes are on a coarsely variegated background they show an appropriately coarse melanophore pattern which is strikingly readjusted to

FIG. 40. A flat-fish, *Paralichthys albiguttus*, on checker-board patterns of different sizes. All figures are from the *same* fish. The length of this fish was 14 cm.; the sides of the checker-board squares were 2 mm., 5 mm., 10 mm., and 20 mm. Mast, Bull. United States Bur. Fish., 1916, 34, pl. 21.

a finely variegated background by a pattern of finer texture (Fig. 40). Lipohumors are represented by the concentrating and the dispersing neurohumors of *Fundulus* and of *Ameiurus*, and by the concentrating neurohumor in *Mustelus*. Thus the two main groups of neurohumors with their numerous representatives promise a rich field for functional investigation.

V

THE NERVOUS SYSTEM AND CHROMATOPHORES

BRÜCKE (1852) in his account of the color changes in the African chameleon compared chromatophores to ordinary muscle and declared that color-cells with their pigment concentrated were in a condition comparable to that of active, contracted muscle. This idea that the phase of concentrated pigment is the active phase of a chromatophore has been accepted by the majority of workers (Keller, 1895; von Frisch, 1912*a*; Spaeth, 1916; Giersberg, 1930; Sand, 1935). Carlton (1903), however, was led to reverse this view for *Anolis* in that he declared that in this lizard the concentrated state was the state of rest. Babák (1913) went still further and expressed the view that both extremes, that of full contraction and of full dispersion, were conditions of activity probably in contrast with some intermediate resting phase.

It is not my intention to discuss this question at length. In fact it would probably be ill advised to do so, for, in my opinion, more work should be done in this general field before a sound conclusion can be reached. Suffice it to say that all the views thus far expressed are based more or less implicitly on a supposed similarity between chromatophores and muscle, especially skeletal muscle. Such a comparison, as I have elsewhere intimated (Parker, 1935*d*), appears to be quite erroneous, and I believe that we should do well in reflecting on the physiology of chromatophores not to let it bias our thoughts.

It is probable that the active states of chromato-

phores are those in which the contained pigment is actually moving in the cells and the quiescent ones those in which it is at rest (Redfield, 1918). There is reason to believe that in what may thus be called the active state of a chromatophore its protoplasm is relatively fluid and mobile, that is, in a sol condition, for the contained melanin particles then show Brownian movement, whereas in what has been called a state of rest the protoplasm is firmer, in a gel state, in which the melanin shows little or no Brownian motion (Gilson, 1926; Parker, 1935*d*). This conception of activity and rest in color-cells is wholly unlike that advanced by the older workers, for it nullifies any comparison between these cells and those of skeletal muscle.

Pouchet's discovery (1876), confirmed by von Frisch (1911), that chromatophores are controlled by what they then called the sympathetic nervous system, but what is now designated the autonomic system, was an important step forward, for it put chromatophores in the category of effectors such as glands and smooth muscle, and removed them from that of ordinary muscle. Spaeth (1916) emphasized this distinction when he declared that chromatophores were modified smooth-muscle cells, and it is certainly true that these two types of tissue have many points in common. The resting and active states of chromatophores as just described are in their essentials very like those of smooth muscle. The active state of this tissue is when its fibers are shortening or elongating. Its resting state is when they are maintaining constant lengths. Smooth muscle is primarily a tonus tissue. Chromatophores may remain weeks in a condition with dispersed or with concentrated pigment, conditions of extreme tonus. But I do not agree with Spaeth in declaring that in consequence of these similarities chromatophores must be regarded as modi-

fied smooth-muscle cells. The sparse and scattered innervation of smooth-muscle cells with only a nerve-terminal here and there in a wealth of cells, is in strong contrast with the multitude of nervous end-organs that surround even a single chromatophore (Fig. 27).

The relation of chromatophores to nerves is extremely diverse. In amphibians these color-cells are probably without direct nervous control and are adjusted entirely through pituitary neurohumors. In the dogfish, *Mustelus*, blanching is a nervous function, and darkening results from a pituitary secretion. In the killifish, *Fundulus*, both blanching and darkening are nervous. If we accept the melanophore system of the killifish with its concentrating and dispersing nerve-fibers as the more usual type, we must turn to other kinds of muscle than smooth muscle for comparison. The best of these is the vertebrate heart-muscle. This muscle, like smooth muscle, is under the control of the autonomic nervous system. Moreover it has a double innervation, sympathetic and parasympathetic. The sympathetic fibers of the heart accelerate its action, the parasympathetic inhibit it. From this standpoint the concentrating fibers of melanophores are believed to come under the same category as the sympathetic fibers of the heart, and the dispersing fibers under the same as the parasympathetic. Much can be said for this comparison, but the heart as a muscle is not the typical tonus organ that the melanophore is. Further the parasympathetic or inhibitory fibers of the heart appear to act on that muscle through acetylcholin, a substance which in all respects fulfills the requirements of a neurohumor and yet appears to have only a very slight effect upon melanophores (Parker, 1934c), an effect which as a matter of fact is the reverse of what was to have been expected. Thus the comparison between chromatophores and heart

muscle is as inadequate as that between these cells and smooth muscle. The truth is that chromatophores, though they have some similarities with smooth muscle and others with heart muscle, differ from both to such an extent that they must be regarded as a type of tissue *sui generis*. They are in no sense to be classed with any kind of muscle.

Is the double autonomic innervation of chromatophores, such as is seen in *Fundulus* and in *Ameiurus*, to be regarded as sympathetic and parasympathetic? This question is not easily answered. It has been studied by Smith (1931a) from the standpoint of the action of drugs. Smith found that cocaine, a stimulus for sympathetic fibers, induced a concentration of pigment in the melanophores of *Fundulus*, and that ergot, a sympathetic depressant, checked this concentration, results which thus favored the view that concentrating fibers belong to the sympathetic division of the autonomic system. In a corresponding way pilocarpin and physostigmin, both parasympathetic stimulants, called forth pigment dispersion and this was retarded by atropin, a parasympathetic depressant. These observations, so far as they go, point to an affirmative answer to the question at the beginning of this paragraph, but experiments with drugs are always precarious, and Smith in his final declaration is cautious not to draw too definite a conclusion.

In the dogfish *Mustelus* only one set of nerve-fibers is present, and they are concentrating fibers. In conformity with what has been said about *Fundulus* these fibers should belong to the sympathetic division of the autonomic system. Following the general concepts of vertebrate neurology they would be classed as post-ganglionic efferent fibers whose cell-bodies lie in appropriate autonomic ganglia and whose axons, as non-

medullated elements, pass out over the gray rami communicantes and the spinal nerves to their peripheral effectors, smooth muscles, glands, or chromatophores. These connections and their functional relations to the melanophores have been recently worked out with great care by Young (1933) in the dogfish *Scyllium*. Here two very significant observations have been made; first, that this dogfish possesses no gray rami communicantes and, second, that it shows no pale areas when its integumentary nerves are cut. This second observation is in strong contrast with what has been described for *Mustelus* by Parker and Porter (1934) and might be taken as ground for doubting the correctness of their statements. A repetition of their work carried out by Parker (1936a) has, however, fully confirmed their findings and incidentally has led to the interesting discovery that the spiny dogfish of the New England coast, *Squalus acanthias*, ordinarily shows no pale bands when the nerves in its fins are cut. In this respect it approaches *Scyllium*. It therefore seems probable that the observations of Young and of Parker and Porter are not really in conflict, but that different species of dogfishes vary in their means of melanophore activation; in some, such as *Mustelus*, the pale phase is under nervous control; in others, such as *Scyllium* and *Squalus*, this phase is induced by other means. Such diversity in a group of even closely related species is not surprising, for, as the study of animal color change progresses, just such individual differences are continually appearing. The conclusion to be drawn from this diversity is that the distinction between sympathetic and parasympathetic autonomic elements which is reasonably clear in the higher vertebrates is by no means so definite in fishes, where individual differences may be very pronounced. In this conclusion I am in agreement with

Young (1931, 1933) whose neurological work on teleosts and elasmobranches points to the inadvisability of drawing these distinctions in the autonomic system of the lowest group of vertebrates with too great sharpness. So far as these two classes of fibers are concerned, the autonomic system in fishes is like a mother liquor out of which has crystallized the much more definite autonomic components—sympathetic and parasympathetic in the higher vertebrates.

The diversity of organization in chromatophoral systems is apparent even more in the variety of neurohumors than in the types of innervations. As I have already pointed out, it would be premature to attempt a general consideration and classification of these activators. The most that can at present be done is to divide them into the two groups of lipohumors and of hydrohumors (Parker, 1935*b*). In *Fundulus*, *Ameiurus*, and *Mustelus* the concentrating agents are lipohumors as are the dispersing humors in *Fundulus* and in *Ameiurus*. The ordinary hydrohumor from the pituitary gland disperses melanophore pigment almost universally; that from the medulla of the adrenal gland, adrenin, concentrates it with still greater uniformity. The parasympathetic fibers to the vertebrate heart produce a hydrohumor, acetylcholin, that inhibits the heart muscle, and the same class of fibers in *Fundulus* produces a lipohumor that disperses melanophore pigment. When we seek for generalizations in such an array of details we find at present little beyond those associated with the two types of solubilities already discussed.

That agents like neurohumors are active in such general central functions as the transmission of nerve impulses from one neurone to another has for some time been surmised. The production of a neurohumor on one side of a synapse and its reception on the other may

well be the explanation of synaptic polarization and of the appreciable loss of time in the passage of an impulse over such a junction. Sir Charles Sherrington in 1925 discussed these problems particularly in relation to central nervous activation and inhibition, and pointed out the possibility of two agents, which he termed A and I, concerned with these operations. Such agents were conceived of even as substances, and the chemical trend thus given to the interpretation of synaptic functions was favored by a number of investigators including Ballif, Fulton, and Liddell (1925), Fulton (1926), and Samojloff and Kisseleff (1927). Others, following the lead of Sir Charles Sherrington (1929), preferred to adopt a less committal attitude and to designate these synaptic conditions as central excitatory states (c.e.s.) and central inhibitory states (c.i.s.) where the term "state" was especially associated with neither action nor substance. (Creed, Denny-Brown, Eccles, Liddell, and Sherrington, 1932.) In this way the unsolved problem of what lies behind these states was temporarily put aside.

So far, however, as melanophores and their responses are concerned, they appear to favor the more strictly chemical interpretation just given to central nervous operations. The concentrating neurohumors of the color-cells would naturally represent the central excitatory substance, and the dispersing chromatophoral neurohumors the central inhibitory substance. Such at least might well be the hypothesized relationships. Central synaptic functions are as a rule strikingly localized. They would therefore be more successfully carried out by lipohumors than by hydrohumors whose tendency to diffuse widely in the watery environment of the central nervous tissues would soon blot out local limitations. Lipohumors have been shown in *Fundulus*

and in *Ameiurus* to occur in opposing pairs. Such pairs with their restricted fields of action may serve as the prototypes of the activating and the inhibiting agents in central nervous operations. Hydrohumors, on the other hand, with their powers of rapid and free spread would exert broad and general influences on the whole nervous organization. Such influences could make themselves felt in the general tone of the central nervous system, the kinds and degrees of personality, and in those abnormal states that fill our hospitals.

In this way neurohumors may play a very significant part in nervous operations. Their occurrence as active intermediaries in the chromatophoral system is a matter of growing certainty. They also appear to have an important rôle in the excitation of smooth muscle and in the control of the vertebrate heart-muscle. It is easy to conceive of them as the effective agents in such central nervous functions as activation and inhibition just mentioned, in the relation of receptor cells to their conducting neurones, and in that large body of nervous interrelations where the integrity of nerve-units is dependent upon the so-called trophic function. In these numerous situations the idea of neurohumors affords interesting hypothetical suggestions that lead at once to experimental tests. The devising and applying of such tests is no easy task, but it is just such exertions that often yield the highest scientific returns. To approach neurohumors and to understand their ways demands the supreme effort of the investigator.

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