COLOR CHANGES IN CRUSTACEANS, ESPECIALLY IN PALAEMONETES

E. B. PERKINS

Zoölogical Laboratory, Harvard University

TWENTY-ONE FIGURES

INTRODUCTION

The comprehensive nature of publications in the field of animal chromatology is attested to by the monographic works of van Rynberk ('06) and of Fuchs ('14), who summarized the literature in a most admirable way. Crustaceans have received no small amount of attention, and although much of the work is a repetition of that done previously, still a wide field has been covered. This includes the older observations on the remarkable power of some shrimps and prawns to change their hues in accordance with the color of the bottom or weed on which they are found; studies on the chromatophores themselves involving their development, structure, and distribution; the problem of pigment formation and migration; and, finally, the physiology of the chromatophores: their reactions to external and internal stimuli, and their control.

It has been shown that the chromatophores of reptiles, amphibians, fishes, and cephalopod molluscs are controlled in some cases by the nervous system and in others by endocrine substances, or both may operate together. Concerning the chromatophores of crustaceans, however, there have been made no such definite conclusions backed with unquestioned experimental evidence.

The first extensive study of crustacean color changes was made by Pouchet in 1872, and the results of his exceedingly careful observations remain unquestioned up to the present time. He showed that changes in color are due to pigment migration within the chromatophores and suggested that they were under the control of the nervous system, although he could not prove this experimentally. He assumed, as did subsequent investigators, that since the eyes are the receptors for the chromatic function, the impulse must be carried over nerves to the chromatophores; but in his attempts to stop color changes by severing nerves he was unsuccessful. Removal of one eye did not interrupt color changes, although removal of both eyes was followed by a lasting expansion of the chromatophores. This was in accord with results subsequently announced by Mayer ('79), Matzdorff ('83), Malard ('93), Menke ('11), Megušar ('12), Degner ('12), and others.

In Matzdorff's work ('83) is found an idea that chromatophores are amoeboid cells whose processes make their way through the hypodermis without having any definite paths prescribed for them. This conception was held as valid for some time, but was subsequently disproved by Keeble and Gamble ('00), by Fröhlich ('10), and by Franz ('10). Matzdorff stated that light does not act directly upon the chromatophores, but only through the eyes affecting the nervous system, and then by way of motor nerves to the chromatophores. The latter view is the one that has been held as the true state of affairs up until the present time.

In the course of his beautiful piece of work on the nervous system of crustaceans, Retzius ('90), using methylen blue, described and figured in Palaemon squilla nerve endings in intimate contact with the chromatophores. Such a condition had never been described previously, nor has it been confirmed since, although many attempts have been made to demonstrate nerve endings.

Keeble and Gamble, and later Gamble alone, in a series of monographs published between the years 1899 and 1910, described in great detail the color physiology of crustaceans in such a thorough way that it would seem at first glance that the last word on crustacean chromatology had been said.

Not only did Keeble and Gamble believe that the nervous system controlled pigment movement, but that light acted directly on the chromatophores by changing their turgor and, consequently, the position of the contained pigment. Bauer ('05) made the same statement after he had observed that blinded animals and isolated pieces of integument showed an expansion of pigment in bright light. It would be well to state at this point in anticipation of the report of the present investigation that such an expansion of chromatophores likewise takes place in total darkness and does not represent the effect of light. Tait ('10) found no direct effect of light upon the chromatophores of the isopod Ligia.

Degner ('12) showed that the ventral ganglionic chain plays no part in the color-change reaction. He concluded that impulses reached the chromatophores by means of peripheral nerves which leave the central nervous system at a high level.

Von Buddenbrock ('25), in the course of his survey of the field of animal chromatology, stated that all chromatophores which we know are under the influence of the nervous system either directly or indirectly. In fishes and cephalopods there are nerves entering the chromatophores, but in other vertebrates and in crustaceans there lacks, he says, such direct innervation. The stimulation here takes place in this manner: as a result of the stimulus and under the action of the central nervous system, a substance is separated from an inner secreting gland into the blood and is distributed to the chromatophores, causing an expansion or contraction of pigment.

This conception of blood control was taken from the work of Koller, which was at that time unpublished and represents the first statement of such a condition in crustaceans. Koller ('25), in his report, announced that the injection of blood from the dorsal vessel of a dark Crangon into one which was white-adapted resulted in a distinct darkening of the latter even on a white background.

The uncertain state of affairs regarding crustacean chromatophores presented a profitable field for research, and at the suggestion of Dr. G. H. Parker, three years ago, I took up the investigation of the physiology of the chromatophores in crustaceans with the purpose of determining especially the nature of the conducting pathways which carry the impulse for color change. I take this opportunity to express my appreciation to Doctor Parker for his friendly criticism and stimulating suggestions.

Although the work was carried on chiefly in the Harvard Zoölogical Laboratory, a great part of it was accomplished at the Laboratory of the United States Bureau of Fisheries at Woods Hole, Massachusetts, where material was collected in abundance. I feel deeply indebted to the Commissioner of Fisheries, Mr. Henry O'Malley, for his ready cooperation in making the facilities there available.

There is lacking any experimental evidence to show that in crustaceans the chromatophores are under the control of the nervous system, and as yet the probability of blood control as announced by Koller has not been confirmed. The investigations recorded in the pages to follow attempt to explain the mechanism of color change in crustaceans, emphasizing especially the effect of the nervous system and of the blood.

EXPERIMENTAL

a. Material

Because of its readily observable color changes and the ease with which it may be collected and cared for in the laboratory, Palaemonetes vulgaris Stimpson was used as the subject for these investigations. This shrimp is a common decapod in the region about Woods Hole and is found clinging to eelgrass and rockweed in brackish or entirely salt water of sheltered coves, bays, and tide pools and often on the bottom in shallow water. In summer it is found in great abundance along the sides of landing stages a few inches below the surface, and by sweeping a dip net rapidly along a float two or three hundred may be gathered in half an hour; in winter, if the water is not frozen, they may be obtained with some difficulty by seining in tide pools and shallow open water.

b. Color changes

On a light sandy bottom these crustaceans are found to be of a light hue matching the surroundings; on a dark mud bottom the animals become correspondingly dark, varying individually from yellowish through reddish to chocolate-brown. In almost any situation of their natural environment these decapods are observed only with difficulty because of their adaptive coloration.

When brought into the laboratory and kept in sea-water, they live very well, provided they are kept cool and the water is changed every two or three days. The stock supply was kept in glass battery jars or in running sea-water when it was available. Animals under observation were in white porcelain dishes and in battery jars painted on the outside with black enamel. In the white dishes they became pale and transparent, while in the black jars they were found to be very dark and in striking contrast to those on the white background (fig. 19). Colored backgrounds were not used, because black or white was more effective in bringing about the extreme conditions desired. By this means the color changes were controlled at will in the course of the experiments.

c. Activity of the chromatophores

Since the work of Pouchet ('72), it has been known that in crustaceans, as well as in fishes, amphibians, and reptiles, the color changes are brought about by the expansion and contraction of chromatophores containing pigment of various colors. In Palaemonetes the processes of the chromatophores contain red or yellow pigment which flows proximally or distally along preformed pathways. This was determined by making a series of photomicrographs of the same chromatophore first expanded, then contracted, and finally expanded again. It was seen that the first and final patterns were the same (figs. 1 to 8), showing that either the pigment flows in and out of fixed channels bounded by walls or the chromatophore as a whole withdraws and extends its processes into preformed spaces in the hypodermis. It is not definitely known which of these two views is true, but the fact remains that the pattern is fixed and the expansion of the chromatophore is not an irregular amoeboid outpushing of processes into the adjacent tissues to form different patterns each time. It should be understood, however, that when the phrase 'contraction of the chromatophore' is used it is done so for lack of a better term and does not imply a state of exact knowledge of the mechanism.

When a shrimp which has become adapted to a black background is put into a white dish, the end result is a complete contraction of all the chromatophores. Accompanying this there is formed, chiefly in the vicinity of the chromatophores, a blue coloration which surrounds them and later permeates the tissues like a dye. It is first seen about two or three minutes after the animal has been placed in the white dish and appears most noticeably at first at the bases of the antennal exopodites, in the heavily pigmented regions over the gills, and finally all over the body. The blue coloration increases in intensity up to an hour and is accompanied by the gradual contraction of the chromatophores. During the second hour the red and yellow pigments become drawn up into their respective centers and the blue disappears. The animal is now pale and transparent and has become completely adapted to the white background. This blue coloration has already been investigated in other decapods by Pouchet ('72), but with the exception of the description of conditions in Hippolyte by Keeble and Gamble ('04), and others, in regard to the nocturnal rhythm nothing further has been done with it. The situation in Palaemonetes and in other crustaceans is not well understood.

In addition to the red and yellow chromatophores and the blue substance, there are seen irregularly and sparsely distributed in the hypodermis symmetrically branched bodies containing a substance which is pale yellow by reflected light and slate gray by transmitted light (fig. 18). They contract and expand in response to backgrounds, but in a reverse direction as compared with the red and yellow chromatophores. On a white background these reflecting yellow chromatophores are expanded into symmetrically branching circular patterns, while on a black background they are contracted to a spherical center. They are of about the same size as the red and yellow chromatophores, but being relatively few in number they take no part in the general coloring of the animal. Very little reference has ever been made to them in other crustaceans. Pouchet ('76) mentioned them and Degner ('12) described white chromatophores in Leander which may be comparable, because they likewise respond in a reverse direction to the other chromatophores. This was further described by Bauer and Degner ('13).

The time necessary for the chromatophores in Palaemonetes to change from one extreme to the other varies with the individual, but the average time is from one to two hours. A noticeable change of hue in the animal as a whole may be seen in from three to five minutes after the beginning of stimulation, this being much more readily observed in the change from dark to light backgrounds, because of the presence of the blue coloration produced under those circumstances. The intensity of the change increases rapidly up to an hour when the animal appears definitely light or dark. During the second hour the pigment masses expand or contract to the extreme condition. Both red and yellow substances migrate at about the same rate.

d. Factors determining color changes

When the chromatophores expand or contract in response to a black or white background, the question is raised as to whether this is a response to differences in light intensity or to the wave length of the light rays emanating from the background. This may be determined readily by subjecting the shrimps in a black jar to a high intensity of light and those in a white dish to a low intensity. It was found that in all cases there was the same degree and rapidity of expansion of chromatophores on a black background and contraction on a white one regardless of the intensity of light.

Keeble and Gamble stated ('04) that in Hippolyte bright light causes an expansion of chromatophores in isolated appendages and pieces of integument, and that in the intact animal this response is inhibited by the nervous system. Bauer ('05) likewise made the same statement for Palaemon and Mysis. It was found in Palaemonetes that the chromatophores of isolated parts do expand in the light, but they expand just as rapidly when the experiment is carried on in total darkness. A box was constructed which was light-proof when tested with sensitized photographic paper. The appendages and pieces of integument were removed in a dark-room and placed in sea-water in the box and left there for varying lengths of time. Pieces were removed at intervals and compared with controls in daylight, and in all cases the rate of expansion was the same for the chromatophores in darkness as for those in daylight. The statement can be made decisively that, in Palaemonetes at least, light does not affect the chromatophores directly.

When the whole animal is placed in absolute darkness, however, there is a complete contraction of pigment to the chromatophore centers regardless of the color of the background on which the animal has previously rested. Animals which had been completely black-adapted were put into a dark-room, and after two hours were found to be pale and transparent, with the chromatophores completely contracted. If the creature had been white-adapted before, it remains so regardless of the time it is kept in the dark. This state of affairs is just the opposite of placing the animal on a black background. In other words, there is a great difference between the effect of black as a background color and the absence of light. In the former case there is produced a complete expansion of pigment, while in the latter there results a complete contraction. It is a peculiar circumstance that darkness should produce the same effect as a white background.

It is obvious that the factor determining the color changes is the reception of various wave lengths of light by the eyes. This was shown by Koller ('25) in Crangon. Pouchet ('72) had shown that at least one eye is necessary for color changes to take place. This was confirmed in Palaemonetes. When one eye is removed by snipping off the stalk at the base, there is no effect upon the chromatic response; but if both eyes are removed, there is a complete expansion of chromatophores regardless of light intensity or color of the background (fig. 19). This complete expansion requires the usual two hours and will likewise take place in the dark. Care must be taken to allow several hours to elapse between the removal of the first and second eyes, because if both eyes are removed at the same time the animal usually dies. Nothing in the way of light intensity or background effect can bring about a contraction of chromatophores after they are once expanded in such blinded animals. On the other hand, if the eyes are blinded by merely painting with a stiff mixture of celloidin and lampblack there is a complete contraction of chromatophores as in absolute darkness. This difference in response between removing and painting the eyes will be explained presently in terms of results obtained from later experiments.

It may be seen that here we have a receptor-effector system with the eyes receiving the stimulus which is carried to the chromatophores as end organs. It has always been assumed that, inasmuch as the eyes are intimately connected with the nervous system, there must be a nervous impulse set up which in some way reaches the chromatophores, although all attempts to prove this influence of the nervous system have met with failure. Nevertheless, for the sake of completeness and confirmation, experiments were planned to show the influence, if any, of the nervous system upon the chromatophores of Palaemonetes.

e. Influence of the nervous system

Anaesthetics. The first attempts were made to nullify any possible effect of the nervous system upon the chromatophores by anaesthetizing the animal with chloretone, magnesium sulphate, chloroform, and ether. It was found that while the animals were under the influence of the anaesthetic there was a complete expansion of chromatophores in white-adapted individuals, whereas the chromatophores of black-adapted individuals remained completely expanded if they were already in that condition, or soon became so if they were not wholly expanded previously. White-adapted animals were placed in white procelain dishes in sea-water and covered with a glass plate. The various anaesthetics were added to the water until the animal stopped all swimming movements and failed to respond to prodding with a glass rod. When chloretone was used, however, the animals were allowed to swim in a 0.2 per cent solution in sea-water until they became anaesthetized. Care was taken to see that the hearts were still beating, and then the animals were removed to fresh sea-water to recover.

The animals became anaesthetized in varying lengths of time, depending upon the anaesthetic used. Ten minutes were usually required for the chloretone, three-quarters of an hour for magnesium sulphate, five minutes for chloroform, and two or three minutes for ether. In all cases the branches of the chromatophores were found to be interlocking to such an extent that it was almost impossible to detect the centers from which they came (fig. 9), and a fine web of color enveloped the whole animal. This condition prevails throughout the period of anaesthesia. Coincident with recovery there is a contraction of chromatophores in response to the background, but there is no formation of the blue substance as when a normal animal is transferred rapidly to a white dish while in the black-adapted condition. If the animal recovers on a black background there is never a contraction of pigment.

It was found that when the animals were heavily anaesthetized with ether the expanded condition persists for hours after locomotion has returned. Several such individuals remained thus for days and could not be distinguished from normal black-adapted specimens. This condition is similar to that seen in animals with the eyes removed, and it was thought that perhaps these animals were blind from the effect of the overdose of ether and, consequently, reacted like blinded animals by presenting a permanent expansion of chromatophores. Various tests showed conclusively that such animals, though active, were really blind. A black rod thrust in front of the animal caused no response, while a normal animal jumped vigorously or brought its antennae rapidly toward the midline of the body. A transparent rod caused no response in normal animals. Moreover, when the animal was put into a deep battery jar it remained near the surface for several seconds and then swam in flat spirals downward until it struck the bottom, whereas the normal animal swam straight downward immediately. It seems unquestionable that such shrimps are blind from the effect of the ether, and this suggests that expansion of chromatophores in all anaesthetized animals is due to a temporary blindness, and not to a direct effect on the nerves leading to the chromatophores.

Attempts were made to anaesthetize locally by minute injections of anaesthetics and by placing crystals of chloretone, magnesium sulphate, and cocaine under the integument, but without success. There was either no effect, general anaesthesia, or the animal died.

Electrical stimulation. If nerves are involved in the color changes, it is reasonable to suppose that the chromatophores could be induced to contract or expand by electrical stimulation. Various parts of the animal were stimulated. This was done by means of a single electrode made from a piece of glass tubing drawn to a capillary point, the other pole being a piece of pure tin immersed in the dish of sea-water containing the animal. The tube was filled with sea-water and the wire from an inductorium was secured to the upper end of the tube and immersed in the water contained within it. The electrode was applied to various regions, such as the eye stalk, nerve cord, heart, blood vessels, body musculature, and digestive tube. There was no effect whatever. The chromatophores failed to respond either by contracting or expanding, although the stimulation was kept up for various lengths of time up to two hours, and with the widest range of currents possible, using a single dry cell and a Harvard coil. Animals used were white-adapted, black-adapted, and blinded individuals. Only when the stimulus was so strong that the animal died soon after did the chromatophores expand, but that is the normal thing as death approaches, no matter what the killing agent may be. A double electrode was also used in order to get a more local stimulus, but this likewise had no effect upon the chromatophores.

Non-polarizable electrodes were then made from two glass tubes, each containing a saturated solution of zinc sulphate with a bar of zinc. The end of each electrode was drawn to a point and packed with kaolin. A single individual was confined in a glass tube, and the electrodes were applied at both ends so that the animal was in the path of the current. After being stimulated on a white background for ten minutes with a current from the inductorium, the chromatophores of a previously white-adapted shrimp were found to be expanded to an intermediate or stellate condition. A control animal similarly confined to a tube, but without electrical stimulation, likewise showed stellate chromatophores, hence it was impossible to determine whether the current had actually stimulated the chromatophores or not. This expansion might be due to lack of oxygen, increase of carbon dioxide, or to a noxious stimulus from contact with the tube.

In order to eliminate the possible effects of decreased oxygen and increased carbon dioxide, it was necessary for the animal to be stimulated electrically in a stream of running sea-water. The apparatus (fig. 15) was a straight glass tube, 15 cm. long and 1 cm. in diameter, open at both ends, with the two non-polarizable electrodes entering at right angles through apertures 7 cm. apart and surrounded by glass sleeves welded into the tube. The whole apparatus was enclosed by white cardboard for background effect. A shrimp could be placed in the tube between the electrodes and held in place by a small piece of glass tubing at each end of the animal resting against the tips of the electrodes. The incurrent water brought in bubbles of air, the amount of air being regulated by adjusting the air inlet. The pressure of water entering the tube could be kept constant for any particular individual by holding the water level in the first glass sleeve at a constant height.

When a white-adapted shrimp was placed in the tube and a stream of sea-water run through, the chromatophores expanded in fifteen minutes to an intermediate condition, and the animal was noticeably darker than when put in. The electric current was then made and the animal stimulated for an hour. There was no further increase of chromatophore expansion. When removed to a white dish where it could swim around freely, there was a slight formation of the blue coloration during white adaptation. The blue stage was likewise passed through when an animal which had reached the intermediate chromatophore condition in a glass tube, without being additionally stimulated electrically, was removed from the tube and placed on a white background.

Other shrimps were placed in the tube successively and nearly all showed an expansion of red and yellow pigments to the intermediate condition whether there was a current of water running through the tube or not. In no case did electrical stimulation either increase or accelerate the expansion, nor did it cause expansion of pigment in those animals whose chromatophores had remained contracted.

The fact that the chromatophores expand in running sea-water eliminates the possibility of decreased oxygen and increased carbon dioxide being the agencies in effecting the color change. The chromatophores, moreover, expand without electrical stimulus, so this expansion is perhaps due to irritation of the animal caused by its cramped position. Pressure against the side of the tube cannot account for the color change, because a small animal in a large tube likewise becomes darker. When a tube is used which is sufficiently large so that the shrimp can swim about freely, there is never an expansion of chromatophores.

Thus, it seems clear that electrical stimulation has no effect upon the chromatophores, and in order to establish evidence for nervous control we must inhibit the impulses for the color changes by actually cutting nerves in the hope of producing a local cessation of the normal chromatic response.

Nerve transection. Central nervous system. That the impulse for color change originates in the eyes is unquestioned; hence it is logical to make successive cuts in different individuals beginning at the eyes and progressing posteriorly along the central nervous system. Attempts to inhibit color changes by cutting the optic nerves, the supra-oesophageal ganglion, the connectives, or the suboesophageal ganglion met with failure, because the animals either died at once or the circulation was interrupted so that death came on later. Posterior to this point it was possible to cut the nerve cord at any level without killing the creature, and if the operation was done so that there was very little loss of blood, the normal color changes went on uninterrupted. After many trials it was found that the whole abdominal nerve cord between the cephalothorax and the telson could be removed by making two slits in the ventral wall at these points so that the nerve cord was severed. Then the lateral segmental nerves were cut with a small hooked scalpel made from a sharpened needle, and the cord was pulled out through one of the slits with fine forceps. When this operation was done neatly and rapidly so that blood clots soon formed over the incisions, it was seen that following this rather serious operation color changes in response to backgrounds went on as in a normal animal. The abdominal segments were, of course, completely paralyzed. In many individuals whose nerve cords had been removed color changes ceased everywhere posterior to the cut end of the attached nerve cord, and it was thought at first that this was due to the severing of nerves which might lead to the chromatophores; but in these cases it could be seen plainly that there was no longer a circulation of blood in the abdomen because of leakage from the ventral sinus. The abdomens of such individuals always became milky white in a few hours, and in a day or two were pink. They had died and were dragged around by the living anterior part of the animal.

It will be recalled that Pouchet ('76), Mayer ('79), Fröhlich ('10), and Degner ('12) had transected nerves in the attempt to inhibit color changes. In Palaemonetes, as Degner found in Leander, extirpation of the eyes from an animal whose nerve cord had been severed or removed was followed by expansion of chromatophores posterior to the cut as well as anterior to it. This indicates that if the chromatophores are under the influence of the central nervous system the impulse must reach them by way of peripheral nerve fibers which leave the cord at a higher level than was possible to reach by cutting. If this were true, it should be possible to sever such nerves by making cuts in the body wall and thus obtain a local cessation of the color changes.

Peripheral nervous system. Many surface cuts several millimeters long were made through the chitinous cuticle and into the body musculature at various places and at many angles in the attempt to sever peripheral nerves which might possibly carry impulses for pigment migration to the chromatophores. In no instance was there the slightest interruption of the normal color changes as a result of making such cuts. Deeper incisions were then made perpendicular to the anteroposterior axis on four sets of animals in such a way that if all the cuts had been made on a single individual its abdomen would have been completely severed. Thus, one cut was made dorsoventrally across the left half of the animal, a similar one on the right side, one across the ventral half going through the nerve cord, and the fourth across the dorsal half severing the dorsal blood vessel. In all except the last case color changes went on normally all over the body. Only in the group of shrimps whose dorsal halves had been cut was there seen a cessation of color changes posterior to the incision. If the chromatophores were expanded before the operation, they remained expanded posterior to the cut when the animal was placed over a white background; and if contracted, they remained so on a black background, although the regions anterior to the cut showed expanded chromatophores. The result was that the shrimp became half white-adapted and half black-adapted. But in all animals operated on in this way the chromatophores posterior to the cut eventually reached an intermediate condition, the tissues became milky white at first and later pink, and the abdomen was seen to be dead.

It is clear that the pathway of the impulse for color changes is in this dorsal region, but if it is along nerve fibers its discontinuity is masked by the necrosis if tissues following transection of the dorsal vessel, and we are unable to determine whether the local cessation of color changes is due to interruption of nervous pathways or not. It will be recalled that in the operations involving removal of sections of the nerve cord many animals were found whose chromatophores failed immediately to respond posterior to the cut. It may be added that this phenomenon occurred when the ventral body wall was opened and the nerve cord left intact, hence it is evident that here, as in the case of the dorsal cut, the interruption of the circulation prevents further expansion and contraction of the chromatophores. Many more cuts were made in the dorsal part until finally it was possible to cut away all the tissues surrounding the dorsal blood vessel, leaving the latter intact, and still have the normal color changes go on. But when this vessel was severed all response of the chromatophores ceased posterior to the cut, although a noticeable change to background could be seen in the parts anterior to the cut in from three to five minutes. Thus, it is clear that the pathway of the impulse for pigment migration is either in or immediately around this blood vessel, or else there is a nerve net carrying the impulse.

Visceral nervous system. In view of the fact that peripheral branches of the central nervous system are not concerned in bringing about color changes, it is logical to suppose that if there is nerve control of the chromatophores it must be by means of the so-called sympathetic or visceral nervous system or by a nerve net. To determine which, if either, of these possibilities is true, several interlocking cuts were made in the free flap of the branchiostegite in such a way that if a stimulus for contraction or expansion reached the chromatophores it would be compelled to travel over a zigzag and tortuous course which a single nerve fiber never would be able to follow. In all cases where the circulation was not interrupted contraction and expansion of the chromatophores went on normally in response to backgrounds even in the remotest part of the maze, and in all cases where the circulation had ceased there were no further color changes. This would lead one to suspect the presence of a nerve net if it were not for the possibility that substances carried in the blood stream might be the determining factors in the control of the chromatophores. The latter possibility will be discussed presently.

In conjunction with physiological methods for indication of nerve control, a histological examination was made for nerve endings in connection with the chromatophores, using methylen blue, and for nerves in the region of the dorsal artery, using vom Rath's fixative. There was no indication in either case of the presence of nerve elements leading to the chromatophores. This, of course, is negative evidence. But considering the rich supply of nerves in the integument as described by vom Rath ('96) and Nusbaum and Schreiber ('97), it is evident that if any of these are concerned in the color-change mechanism they would have been severed by the cuts, and the color changes accordingly stopped. Nusbaum ('99) demonstrated ganglion cells and a nerve net in the heart of Palaemon and figured a portion of the abdominal aorta with an elaborate supply of nerve fibers; and Alexandrowicz ('09, '13) described nerve fibers running under the integument and along the intestine and blood vessels. Consequently, it is seen that, although many nerves are present, there is no evidence that they lead to the chromatophores.

f. Influence of the blood

From evidence obtained by anaesthetizing, by electrical stimulation, by cutting, and by histological studies it is clear that if nerves play a part in bringing about color changes they serve only to initiate the process and do not represent the pathways over which the impulse for the chromatic response is carried. One is forced to suspect that the activity of the chromatophores is brought about, ultimately at least, by some substance or substances carried in the blood stream. No direct proof of hormone control of vital processes in the invertebrates had been demonstrated up to the present time, although during the course of this research there was published by Koller ('25) a preliminary report describing the effect of injecting blood from a dark shrimp (Crangon) into the vessels of a light one, producing thereby a darkening of the latter. This is the first recorded instance of such a phenomenon outside the vertebrates. At the time Koller's report was published, I had injected blood from one shrimp into another without inciting color change, and up to the present further trials have met with no success. Injection of the vertebrate hormones adrenin and pituitrin were without effect on the chromatophores except when the amount introduced was so great that there was pigment expansion as a result of death.

During the search for nervous pathways for color changes it was found that the only cut which interrupted the phenomenon was one through the dorsal abdominal artery, thus localizing the course of the impulse to the blood vessel or to a nerve net. Other arteries were now severed to determine if a local cessation of the color response was met with, and it was found that cutting the right or left antennary artery resulted in a disturbance of the chromatic function in the regions supplied by those arteries (figs. 11, 12), while section of the median ophthalmic artery produced complete expansion of chromatophore pigments all over the body, as in the case of shrimps whose eyes have been removed. The latter situation is suggestive of the important rôle of the eyes in the color-change mechanism.

Two objections may be raised to this procedure of cutting. The first is that the tissues are deprived of blood and the chromatophores might not function, even though they had the proper stimulus, because of the accumulation of carbon dioxide, lack of oxygen, changes in the hydrogen-ion concentration, and general metabolic disturbances. After an artery is cut on a shrimp which has become black-adapted and the animal is then placed on a white background, all the chromatophores except those supplied by this artery start to contract in from three to five minutes, as evidenced by the formation of the blue pigment, while those in the operated region show no sign of contracting. It seems reasonable to suppose that the chromatophores could start to contract within five minutes after the blood supply has been cut off, so far as the condition of the tissues is concerned, were they under the control of the nervous system. Bennitt ('24) found that the retinal pigment in the eyes of Gammarus would respond to stimuli one or two hours after removal of the eye from the body. Though not strictly comparable, it may be inferred that the chromatophores of Palaemonetes likewise would be able to contract when isolated from the body, provided they were properly stimulated. The second objection is that nerves which might be running either in the wall of the blood vessel or closely associated with it and conducting the impulse for color changes would be severed.

To overcome these objections attempts were made to ligate the antennary arteries with a single silk fiber so that the circulation could be cut off without cutting nerves. The technical difficulties involved, however, were so great that the animal died from loss of blood from the surrounding blood spaces, inasmuch as the wound was kept open in the process of manipulating the fiber. According to Yung ('78), the heart is supplied by stomatogastric nerves which run closely attached to the ophthalmic artery, and by fibers arising from the thoracic ganglia. Injury to these nerves would cause the death of the animal. Another objection arose in this connection, namely, that if the animal survived the operation, the fact that color changes ceased in the regions supplied by the blood vessel might indicate a nerve block due to pressure of the fiber around the blood-vessel wall. The dorsal abdominal artery was next ligated and color changes stopped posterior to the ligature, but here again, due to loss of blood from the dorsal sinus, the animal died before the fiber could be untied. There was also great possibility of breaking the blood vessel during the tying and untying of the ligature. The method was altogether unsatisfactory and, due to the extremely small size of the artery, the task of clamping it was found to be an exceedingly difficult one.

Finally, a comparatively simple technique was developed by which the artery could be occluded in less than thirty seconds and released without injury in about five seconds, all with practically no loss of blood. With fine iridectomy scissors a V-shaped cut was made in the side of one of the abdominal segments so that a triangular flap of the shell resulted. Then fine iris forceps were forced in through the flexor muscles and the intestine and blood vessel, which are closely attached to each other, were pulled out together and looped over the flap of shell (fig. 16). They are sufficiently elastic to allow for this without breaking. This resulted in a pinching off of the blood vessel in two places, one where it left the body and the other where it entered again. By pushing the loop of intestine and blood vessel farther up into the wedge of shell, greater pressure could be put on the vessel in case that were needed to occlude it. Ordinarily, the mere process of looping the vessel over the flap was sufficient to stop all circulation posterior to this point, as could be seen by noting the movement of corpuscles in the blood vessel. The artery was released and the circulation restored by snipping off the flap and pulling out the triangular piece of shell under the artery.

When such a loop was made on a black-adapted shrimp and the creature transferred to a white dish, the parts anterior to the loop became white-adapted, while those posterior to it remained dark. When the artery was released by snipping off the flap, the circulation was restored and the chromatophores contracted in the whole abdomen posterior to the loop. When the operation was carried out on a white-adapted animal, which was immediately transferred to a black jar, the parts anterior to the loop became dark, while those posterior to it remained light with the chromatophores withdrawn tightly into minute dots. After an hour the loop was released and the chromatophores became as much expanded posterior to the loop as anterior to it.

It may be shown histologically and physiologically that in Palaemonetes, contrary to the condition found in other decapods, there is no sternal artery and no ventral abdominal artery. Thus, occlusion of the dorsal abdominal artery affects all the chromatophores in the abdomen posterior to the occluded region, because the entire abdominal circulation is interrupted. This peculiar situation is not mentioned by Bouvier ('91), who stated quite definitely that all the decapods, with the exception of Pagurus, possess two abdominal arteries, a superior and an inferior.

Even after the tissues have been without a circulation of blood for an hour and more, the chromatophores regain the ability to expand and contract in response to backgrounds upon return of the circulation, hence one of the objections to cutting the artery is eliminated. It is now clear that if the chromatophores in the region supplied by the cut artery were under the control of the nervous system they would be able to function as far as their state of metabolism is concerned, provided they receive the proper stimulation. But there is still the objection, of course, that they may be innervated by nerves running either in or closely applied to the walls of the blood vessels and following them everywhere throughout the ramifying system.

Fortunately, many animals were found after such an operation in which the circulation had become only very poorly reestablished, either because of leakage or from a kink in the vessel, so that the blood pressure posterior to the loop was exceedingly low. In these cases of imperfect reestablishment of the circulation there was a corresponding delay in the chromatic response and then seen only in the uropods and telson, while the rest of the abdomen posterior to the loop remained dark. This delayed and localized response may be interpreted as due to lack of sufficient pressure to force the blood into the finer segmental arteries, but enough to carry it through the larger dorsal artery to the posterior end of the animal. Microscopic examination revealed that there was a slow and somewhat irregular circulation in the uropods and telson continuous with that of the dorsal artery, but no circulation in the fine lateral branches of the artery. Such cases as these show conclusively that a nerve block caused by pressure on the blood vessel could not have been the reason for the failure of the abdomen to change color after the blood vessel was occluded.

When a white-adapted shrimp which was operated on in this way was transferred to a black jar, the parts anterior to the loop became dark in the usual time, while those posterior to it remained light with the pigments withdrawn into the chromatophore centers, as has been described previously. This animal was then returned to the white dish and the loop released, so that the anterior dark half of the animal, carrying with it the factor for expansion of the chromatophores, was permitted to flow back into the posterior region of contracted chromatophores. It might be expected that these contracted chromatophores could be induced to expand, even on a white background, due to influence of blood from the anterior dark half much the same as when blood is injected from a dark animal into a light one. Such was found to be the case. Upon the return of the circulation, the contracted chromatophores began to expand gradually until about fifteen minutes later, when a state of equilibrium was reached. The posterior parts were distinctly darker than is ever seen in a similar abdomen with no circulation. Beginning at this time, however, the whole animal was seen to be noticeably paler, due to the influence of the white background, and in an hour or more it had become quite adapted to the background.

The reverse process could be carried out by occluding the artery on a dark animal, causing the anterior parts to become light on a white background, and releasing the circulation on a black background. The backward-rushing blood carried the factor for contraction and the chromatophores responded accordingly, though they were on a black background. They later expanded after an equilibrium had been reached.

Attempts were made to inject blood from one individual into another, but without success, because of rapid coagulation.

These experiments upon the circulatory system reveal clearly that the coordinating means intervening between the eyes and the chromatophores is a substance or substances carried in the blood stream, and by its activity there is brought about proximal and distal migration of pigment within the chromatophores. When cut off from the blood supply, the chromatophores remain for several hours in the same degree of expansion or contraction as when isolated, finally reaching an intermediate condition of expansion which is presumably a state of rest, and remain thus until they disintegrate (fig. 10).

g. The eyes as centers of activation for the color-change mechanism

Having determined that the factor for bringing about pigment migration is carried in the blood stream, it is important next to know where and how this substance is formed. As far as is known, there are no endocrine organs in the invertebrates comparable to the adrenal and pituitary bodies in the vertebrates, which might possibly produce a substance regulating chromatophore activity, yet there must be some seat of formation of such material here. Attempts were made to locate such a center by crushing different parts of Palaemonetes in sea-water and injecting the resulting extracts into black-adapted, white-adapted, and blinded animals. Extracts were made of central nervous tissue, muscle, gonad, and digestive tract, but with no effect upon the chromatophores.

It might be supposed that, inasmuch as the eyes are essential for the functioning of the color changes, they perhaps serve as endocrine organs under certain conditions of light stimulation. Consequently, several eyes clipped from white-adapted individuals were crushed together in sea-water and the resulting fluid injected into the dorsal sinus of a blinded animal whose chromatophores were permanently expanded. Previously, all attempts to cause contraction of such chromatophores had ended in failure. Now, however, there came on within a few minutes a striking contraction of these hitherto refractory chromatophores together with a formation of the blue coloration which is so characteristic of the change from the dark to the light condition. Within an hour, this blinded animal was nearly as light as one which is normally white-adapted (fig. 21). This period of contraction lasted for approximately twenty-four hours, when the chromatophores again expanded to their previous condition. Other blinded shrimps were injected with sea-water to serve as controls, but there was no effect upon the chromatophores.

Another control individual was injected with an extract of eyes from black-adapted animals and showed a slight contraction of chromatophores which lasted for less than half an hour, and with the formation of only a small amount of the blue coloration. This indicates that the substance bringing about contraction of the chromatophores is formed by the activity of light upon the eye, especially when the injection of eye extract which had been left in the light for several minutes before injection brought about a further degree of contraction than was seen following immediate injection. Similar contraction of chromatophores could be induced by injection of eye extracts into seeing black-adapted animals kept over a black background, in spite of the antagonistic effect of the background through their own eyes.

It was found that extracts of eyes from either white-adapted or black-adapted animals failed to bring about expansion of chromatophores in white-adapted shrimps. What, then, causes the chromatophores to expand? If there is a second substance specific for chromatophore expansion, it does not assert itself upon being injected into the tissues. If there is only one substance whose presence brings about contraction, its absence does not allow for expansion, because contracted chromatophores remain in that condition for many hours when deprived of the circulation or after being isolated from the rest of the body. Thus, varying amounts of some one substance formed in the eyes cannot account for all the degrees of expansion and contraction of the chromatophores, for expansion of chromatophores in the normal animal is an active process taking only an hour or two for its completion and continuing regardless of the intensity of light.

The fact that the substance bringing about the contraction of the chromatophores has its origin in the eyes gives us an explanation for the state of extreme expansion seen when the eyes are removed. Under such conditions, the center of formation of the substance responsible for contraction is gone and the factor for expansion is allowed to assert itself to the fullest. When the eyes are merely blinded by coating with an opaque mixture, the factor for contraction is still present and dominates over the factor for expansion under these particular conditions. Just why blinding in this way, absolute darkness, and white backgrounds all bring about a contraction of chromatophores is a question which as yet has not been satisfactorily answered.

Further work along this line is certain to bring out more clearly facts concerning endocrine organs in the invertebrates. The evidence presented here indicates that the eye stalks are in reality such structures, and there is opened up a field of investigation which is not without a certain amount of importance.

SUMMARY

1. Palaemonetes vulgaris Stimpson was used as a subject for the investigation of color changes.

2. These decapods change their hues in accordance with the color of their surroundings. They become reddish brown on a dark background; pale and transparent on a light one. The adaptation is sufficient to afford a certain degree of concealment.

3. The change from the light to the dark condition is effected by change in shape of chromatophores containing red and yellow pigments. In the contracted condition the chromatophores are drawn up into minute spherical masses so that the color of the animal is that of the great mass of its tissues; in the expanded condition the red and yellow pigments have migrated distally from the chromatophore centers to form branching figures, and this results in a network of color enveloping the whole body.

4. The pattern of the chromatophore is fixed, successive periods of expansion following intervals of contraction always showing the same design.

5. Accompanying the change in color when a shrimp is transferred from a black to a white background there is formed, chiefly in the vicinity of the chromatophores, a blue substance which surrounds them and later permeates the tissues like a dye. This blue coloration is transitory, disappearing within two hours.

6. Chromatophore-like bodies which are pale yellow by reflected light and slate-gray by transmitted light are seen irregularly and sparsely distributed in the hypodermis. They contract when the red and yellow chromatophores expand, and expand when these contract.

7. The time of change from one extreme of color to the other is approximately two hours, varying with the individual.

8. In Palaemonetes light does not act upon the chromatophores directly, but only by mediation of the eyes.

9. Absolute darkness and coating the eyes with an opaque mixture bring about a complete contraction of the chromatophores regardless of background or light conditions.

10. Removal of one eye has no effect upon the chromatophores; removal of both eyes brings on a lasting expansion which is unaffected by light or background.

11. Anaesthetics cause an expansion of chromatophores. This is probably due to temporary blindness.

12. I was unable to change the chromatophores by electrical stimulation.

13. Nerve transection does not interfere in any way with the color responses.

14. It was demonstrated that peripheral and visceral nerves, or a nerve net, are not concerned with the expansion and contraction of the chromatophores.

15. There is no histological evidence to show that the chromatophores are innervated.

16. Occlusion of an artery always results in a cessation of the chromatic response in the region supplied by that artery. This is believed to be due to lack of a substance carried in the blood stream. Upon release of the vessel, the region formerly deprived of blood takes on the color of the rest of the animal.

17. An extract of the eye stalks of Palaemonetes when injected into blinded animals brings about a contraction of the hitherto refractory chromatophores. Extract of eyes from white-adapted shrimps induces a greater and more lasting contraction than that from black individuals.

18. There is evidence to support the statement that the eyes are probably endocrine organs producing a substance which, carried to the chromatophores by the blood stream, brings about a contraction of the chromatophores.

19. If there is a second substance specific for chromatophore expansion, it does not assert itself upon being injected into the tissues. There is no evidence to indicate that expansion of chromatophores is a result of tonus or deficiency of substance bringing about contraction.

LITERATURE CITED

ALEXANDROWICZ, J. S. 1909 Zur Kenntnis des sympathischen Nervensystems der Crustaceen. Jenaische Zeitschrift für Naturwissenschaft, Bd. 45, S. 395–427.

——— 1913 Zur Kenntnis des sympathischen Nervensystems einiger Wirbellosen. Zeitschrift für allgemeine Physiologie, Bd. 14, S. 358–376.

BAUER, V. 1905 Ueber einen objectiven Nachweis des Simultancontrastes bei Tieren. Centralblatt für Physiologie, Bd. 19, S. 453–462.

BAUER, V., UND E. DEGNER 1913 Über die allgemeinphysiologische Grundlage des Farbenwechsels bei dekapoden Krebsen. Zeitschrift für allgemeine Physiologie, Bd. 15, S. 363–412.

BENNITT, R. 1924 The migration of the retinal pigment in crustaceans. Jour. Exp. Zoöl., vol. 40, pp. 381–435.

BOUVIER, E. L. 1891 Recherches anatomiques sur le système artériel des crustacés décapodes. Annales des Sciences-Naturelles, T. 11, pp. 197–282.

BUDDENBROCK, W. VON 1925 Grundriss der vergleichenden Physiologie. Zweiter Teil: Atmung, Effectoren, S. 388–412. Berlin.

DEGNER, E. 1912 Über Bau und Funktion der Krusterchromatophoren. Eine histologisch-biologische Untersuchung. Zeitschrift für wissenschaftliche Zoologie, Bd. 102, S. 1–78.

FRANZ, V. 1910 Zur Struktur der Chromatophoren bei Crustaceen. Biologisches Centralblatt, Bd. 30, S. 424–430.

FRÖHLICH, A. 1910 Farbwechselreaktionen bei Palaemon. Archiv für Entwicklungs-mechanik der Organismen, Bd. 29, S. 432–438.

FUCHS, R. F. 1914 Der Farbenwechsel und die chromatische Hautfunktion der Tiere. Hans Winterstein's Handbuch der vergleichenden Physiologie, Bd. 3, Hälfte 1, zweiter Teil, S. 1285–1368. Jena.

GAMBLE, F. W. 1910 The relation between light and pigment-formation in Crenilabrus and Hippolyte. Quarterly Journal of Microscopical Science, vol. 55 (New Series), pp. 541–583.

GAMBLE, F. W., AND F. W. KEEBLE 1900 Hippolyte varians: a study in colour-change. Quarterly Journal of Microscopical Science, vol. 43, pp. 589–698.

KEEBLE, F. W., AND F. W. GAMBLE 1899 The colour-physiology of Hippolyte varians. Proceedings of the Royal Society of London, vol. 65, pp. 461–468.

——— 1903 The colour-physiology of higher Crustacea. Proceedings of the Royal Society of London, vol. 71, pp. 69–71.

——— 1904 The colour-physiology of higher Crustacea. Philosophical Transactions of the Royal Society of London, Series B, vol. 196, pp. 295–388.

——— 1905 The colour-physiology of higher Crustacea. Part III. Philosophical Transactions of the Royal Society of London, Series B, vol. 198, pp. 1–16.

KOLLER, G. 1925 Farbwechsel bei Crangon vulgaris. Zoologischer Anzeiger. Verhandlungen der Deutschen Zoologischen Gesellschaft E. V., S. 128–132.

MALARD, A. E. 1893 The influence of light on the coloration of crustaceans. Annals and Magazine of Natural History, vol. 11, 6th series, pp. 142–149.

MATZDORFF, C. 1883 Ueber die Färbung von Idotea tricuspidata. Jenaische Zeitschrift für Naturwissenschaft, Bd. 16, S. 1–58.

MAYER, P. 1879 Ueber Farbenwechsel bei Isopoden. Mittheilung aus der zoologischen Station zu Neapel, Bd. 1, S. 521–522.

MEGUŠAR, F. 1912 Experimente über den Farbwechsel der Crustaceen. (I. Gelasimus; II. Potamobius; III. Palaemonetes; IV. Palaemon.) Archiv für Entwicklungs-mechanik der Organismen, Bd. 33, S. 462–665.

MENKE, H. 1911 Periodische Bewegungen und ihr Zusammenhang mit Licht und Stoffwechsel. Archiv für die gesammte Physiologie, Bd. 140, S. 37–91.

NUSBAUM, J. 1899 Beiträge zur Kenntnis der Innervation des Gefässsystems nebst einigen Bemerkungen über das subepidermale Nervenzellengeflecht bei den Crustaceen. Biologisches Centralblatt, Bd. 19, S. 700–711.

NUSBAUM, J., UND W. SCHREIBER 1897 Beitrag zur Kenntnis des peripherischen Nervensystems bei den Crustaceen. Biologisches Centralblatt, Bd. 17, S. 625–640.

POUCHET, G. 1872 Sur les rapides changements de coloration provoqués experimentalement chez les crustacés et sur les colorations bleues des poissons. Journal de l'Anatomie et de la Physiologie, T. 8, pp. 401–407.

——— 1876 Des changements de coloration sous l'influence des nerfs. Journal de l'Anatomie et de la Physiologie, T. 12, pp. 1–90, 113–165.

RATH, O. VOM 1896 Zur Kenntnis der Hautsinnesorgane und des sensiblen Nervensystems der Arthropoden. Zeitschrift für wissenschaftliche Zoologie, Bd. 61, S. 499–539.

RETZIUS, G. 1890 Zur Kenntnis des Nervensystems der Crustaceen. Biologische Untersuchungen, Neue Folge, Bd. 1, S. 1–50. Stockholm.

RYNBERK, G. VAN 1906 Ueber den durch Chromatophoren bedingten Farbenwechsel. Ergebnisse der Physiologie, Bd. 5, S. 397–428.

TAIT, J. 1910 Colour change in the isopod, Ligia oceanica. Journal of Physiology, vol. 40, pp. XL–XLI of the Proceedings of the Physiological Society.

YUNG, E. 1878 Recherches sur la structure intime et les fonctions du système nerveux central chez les crustacés décapodes. Archives de Zoologie Expérimentale et Générale, T. 7, pp. 401–534.

EXPLANATION OF PLATES

All figures are from Palaemonetes vulgaris

PLATE 1

EXPLANATION OF FIGURES

1 Expanded chromatophore.

2 The same chromatophore as that shown in figure 1 beginning to contract.

3 After thirty minutes on a white background.

4 The same chromatophore nearly contracted in response to stimulus from a white background.

5 The same chromatophore completely contracted after the shrimp had been on a white background for two hours.

6 Beginning to expand over a black background.

7 After an hour on a black background. A blood vessel may be seen in the upper right.

8 The same chromatophore after two hours on a black background. Comparison with figure 1 shows that the chromatophore returns to the same pattern after it has contracted.

PLATE 1

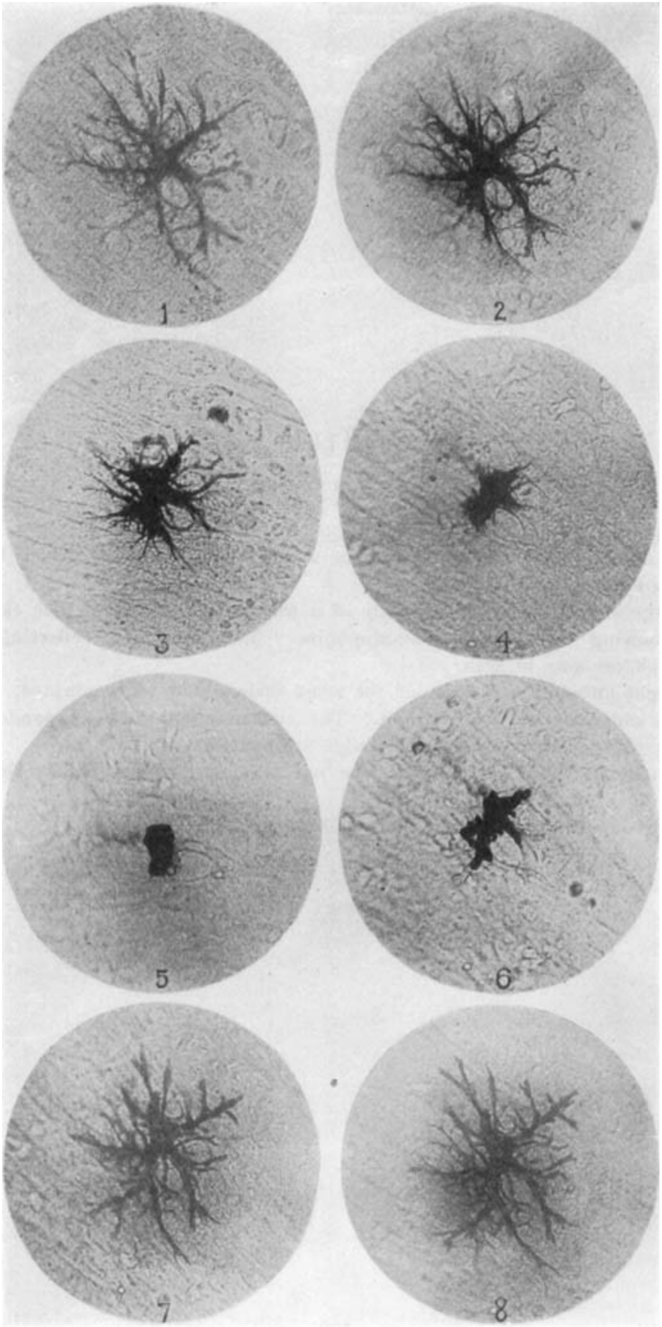

PLATE 2

EXPLANATION OF FIGURES

9 Interlocking branches of two adjacent chromatophores.

10 A disintegrated chromatophore in a portion of the body which has been deprived of blood for forty-eight hours.

11 Edge of left antennal exopodite of a white-adapted shrimp with circulation intact, showing contracted chromatophores. Two expanded reflecting yellow chromatophores may be seen.

12 Right antennal exopodite of the same shrimp still white-adapted, but with the right antennary artery severed. The chromatophores are expanded. The reflecting yellow bodies on the right edge are contracted.

13 Contracted chromatophores. The red pigment appears black; the yellow stippled.

14 A group of expanded chromatophores.

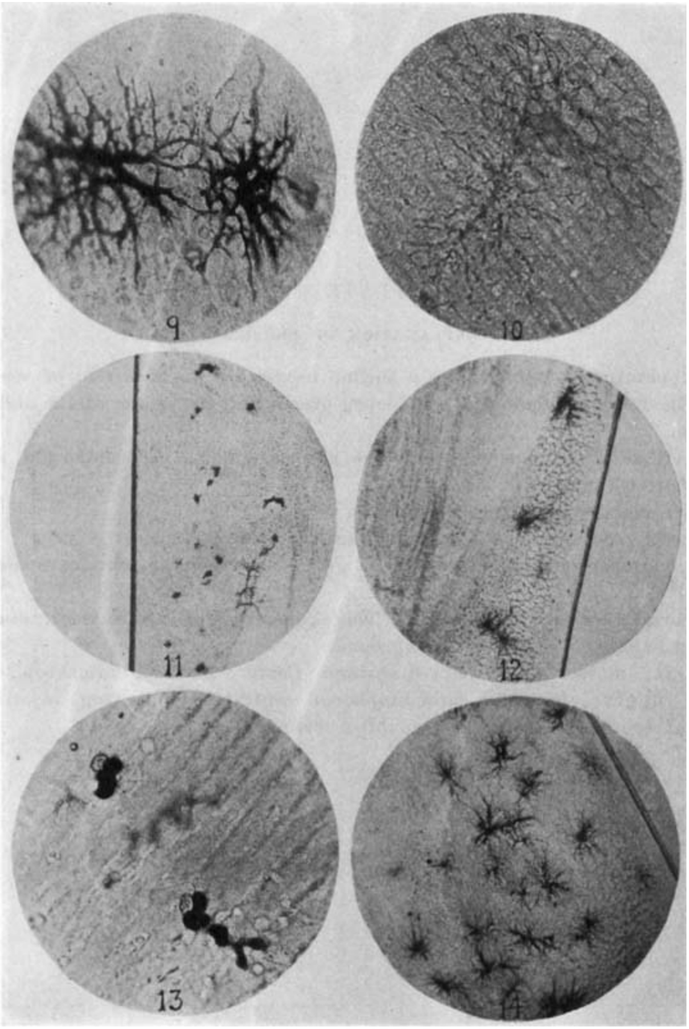
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PLATE 3

EXPLANATION OF FIGURES

15 Apparatus for stimulating a shrimp electrically in a current of sea-water.

16 The dorsal abdominal artery looped over a flap cut in one of the abdominal segments.

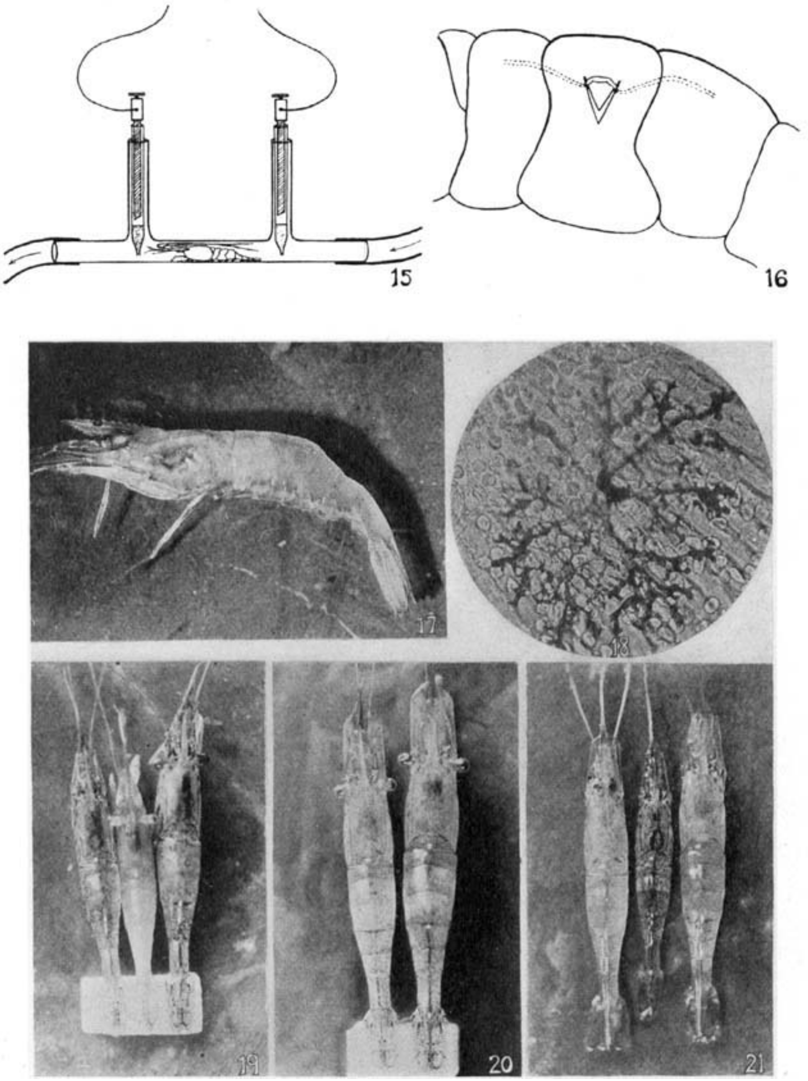
17 Left side of Palaemonetes. The chromatophores are contracted as seen over a white background.

18 A reflecting yellow chromatophore.

19 Left: both eyes removed, chromatophores expanded. Center: white-adapted, chromatophores contracted. Right: black-adapted, chromatophores expanded.

20 Dorsal view of Palaemonetes. The chromatophores are in the intermediate condition as seen over a gray background.

21 Left: normal white-adapted shrimp. Center: blinded, chromatophores expanded. Right: blinded, chromatophores contracted following injection of extract of eye stalks from white-adapted shrimps.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