SCIENCE

VOL. 92 FRIDAY, OCTOBER 11, 1940 No. 2389

SCIENCE: A Weekly Journal devoted to the Advancement of Science, edited by J. MCKEEN CATTELL and published every Friday by

THE SCIENCE PRESS

Lancaster, Pa. Garrison, N. Y.

New York City: Grand Central Terminal

Annual Subscription, $6.00 Single Copies, 15 Cts.

SCIENCE is the official organ of the American Association for the Advancement of Science. Information regarding membership in the Association may be secured from the office of the permanent secretary in the Smithsonian Institution Building, Washington, D. C.

A MODERN CONCEPTION OF THE ACTION OF THE NERVOUS SYSTEM[1]

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IT was my privilege as a young student of zoology to have made the acquaintance of Professor W. K. Brooks in the summer of 1889 at the Laboratory of the United States Fish Commission, Woods Hole. Here a body of mature investigators in marine biology was gathered and we younger workers were allowed to associate with them greatly to our advantage. It is an honor to be invited by Miss Fowler to deliver this lecture, and it would be a pleasure to me if I could think of it as a small return to Dr. Brooks for the kindly help and encouragement he gave to all of us who were associated with him at the Woods Hole Station. In a way this lecture is an appropriate tribute to Dr. Brooks, for much of its contents was brought to light in those laboratories that have grown up at Woods Hole around the original one where he worked. I am further disposed to think that the special subject herein discussed, the mode of interaction of nervous elements, would have claimed a fair share of Dr. Brooks's interest, for his philosophical temperament would have led him not only to seek a clear picture of the nervous mechanism in animals, but to gain an insight into the way in which this mechanism acts.

The histologists of half a century ago described the nervous system as composed of ganglion-cells, nerve-fibers and fibrillar material. With the advent of the Golgi method in the last quarter of the past century it became possible to determine the relations of these

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[1] Third William Keith Brooks Lecture, delivered at Greensboro College, Greensboro, N. C., April 25, 1940.

elements to each other. Golgi's idea that the fibrillar material forms a true network was abandoned for Ramòn-y-Cajal's conception of the fibrillar substance as a system of branches by which one nerve-cell is brought into contact with others but without real unions. This separateness of the nerve-cells was foreshadowed in the embryological studies of His and was abundantly confirmed subsequently by the histogenetic investigations of Harrison. Meanwhile Waldeyer in 1891 had seized upon this revised histological picture of the nervous system, and, recognizing that nerve-fibers were parts of nerve-cells, had been led to the conception of the true histological units or neurones whose functional continuity was through fibrillar contacts. These points of mutual contact, the synapses, were shown by Sherrington to have a kind of transmission unlike that of a nerve-fiber. They proved to be polarized in that they transmitted nerve impulses in one direction only as contrasted with the double conduction in nerve-fibers. Synaptic transmission moreover was more time-consuming than fiber transmission. Many neurophysiologists have interpreted synaptic transmission as a purely electrical phenomenon, but of recent years there has been a growing opinion that synaptic transmission is accomplished by substances, variously designated as neurohumors, neurohormones, or chemical activators, which, produced on one side of the synapse, pass across the minute interruption and stimulate the opposite side. The chief question to be discussed in this lecture is whether there is evidence for the existence of such substances and what their natures may possibly be.

Such substances may be sought between receptor-cells and their associated nerve-fibers, or where one neurone is in contact with another in nervous organs, or finally where efferent neurones terminate on effectors such as muscles, glands, color cells and the like. It is to the last set of these contacts, those between chromatic nerve-fibers and their associated color cells or chromatophores that I propose to direct your attention.

By means of chromatophores lodged for the most part in the skins of many animals these creatures can change their color in many directions and often with astounding quickness. These changes were well known to the ancients and were recorded by Aristotle and by Pliny. They have excited the attention of naturalists down the ages. Such changes are to be seen among squids and devil-fishes, shrimps and prawns, and among the lower vertebrates including the fishes, the amphibians, and the lizards. Incidentally many other animals show such changes, but the best-known examples are found among the groups just indicated. For conciseness I shall limit my discussion to the chromatic activities of the lower vertebrates.

If a smooth dogfish, Mustelus, be placed in an illuminated marine aquarium with white sides and white bottom, it will assume in the course of a few days a pale, translucent flesh-tint. Placed now in a similar black-walled tank, it will become in a day or so deep gray. These color changes from dark to pale and the reverse constitute the whole color range of this fish and are accomplished by the concentration or dispersion of a dark pigment, melanin, in myriads of chromatophores in the skin of this fish. In the concentrated state the chromatophores or, better, melanophores are black dots each about 30 microns in diameter; in the dispersed state they are richly branched, dark bodies each covering an area roughly 200 microns in diameter. As dots they are inconspicuous in the total area of the skin and induce the pale tint in the fish, but as branched bodies they together cover large areas of skin and bring about the dark coloration. This relatively simple mechanism illustrates the device by which most lower vertebrates change their tints though in many of these animals their melanophores are supplemented by chromatophores of other colors, red, orange, yellow, white, and the like.

In 1858 the celebrated English physician Lister studied the color changes of the common frog and made the discovery that the integrity of the eyes in this animal was necessary for its chromatic activities. He concluded that these activities were in the nature of nervous reflexes and were mediated by the central nervous system of the animal. Pouchet, in a series of papers published between 1872 and 1876, showed that in fishes the parts of the nervous system especially concerned with color changes were the sympathetic or autonomic tracts. In the first quarter of this century Sumner and subsequently Mast demonstrated that certain fishes, the flatfishes or flounders, not only changed their general tint but also modified their color patterns to fit in a rough way that of their surroundings. This change of pattern may be regarded as perhaps the most complicated of the color changes in any animal and represents in this respect the pinnacle of efficiency in such chromatic systems.

Up to about 1920 all investigators in this field of research accepted the nervous interpretation of the control of animal color changes, but beginning with 1922 a novel view concerning such changes was advanced. In that year Hogben and Winton showed by a variety of tests that the nerves in frogs had little or nothing to do with the color changes in these animals, but that such changes were dependent upon an internal secretion from the pituitary gland. A frog from which this gland had been removed became pale and remained so. On injecting into such a pale animal an extract of the intermediate lobe of this gland it was observed that the creature became very dark but blanched again after time had been given for the extract to have disappeared. From this and other like tests Hogben concluded that amphibians became pale when the pituitary secretion, now called intermedin, was absent from their blood and that they were dark when this substance was present in their circulation. He was thus led to contrast the hormonal control of color changes in amphibians with the nervous control of these changes in the fishes and in the lizards. Hence so far as the color changes of amphibians were concerned an entirely novel element was introduced. To discuss the importance of hormonal or humoral agents in color changes I propose to consider the chromatic activities in three lower vertebrates, the frog, the smooth dogfish, and the catfish.

The chromatic system in the frog so far as activation is concerned may well represent the simplest type of this process. As I have already mentioned, Hogben and Winton expressed the view that the color changes in the frog were fully explicable from the standpoint of the presence or absence of a single chromatic secretion. This opinion was espoused by Parker and Scatterty. Meanwhile Hogben and Slome in their study of the South African toad Xenopus were led to conclude that amphibians possessed a second and in this instance a blanching neurohumor also from the pituitary gland, a conclusion supported by the work of Soderwall and Steggerda on frogs. Whether amphibians possess one, two or more chromatic hormones is an open question, but however this may be, it is agreed by all recent physiological workers that melanophore activation in the frog is humoral and not nervous. The innervation of frog melanophores as shown in histological preparations by some recent students of this subject (Perotti, Tusques) has in no instance been demonstrated to be concerned with color changes and may well have to do with other nervous functions. A purely humoral activation of chromatophores appears to hold not only for amphibians, but also for the lamprey eels (Young), most sharks and rays (Young, Parker, Wykes), the Carolina lizard (Kleinholz), and the shrimps, prawns, crabs and the like (Perkins, Koller, Abramowitz, Hanström, Carlson, Brown, Kleinholz and others).

A second type of chromatic activation is to be seen in the smooth dogfish Mustelus. Like the frog this fish becomes pale on the loss of its pituitary gland and darkens again temporarily to an injection of pituitary extract (Lundstrom and Bard). When its cutaneous nerves are cut the denervated areas become pale, and the electric stimulation of cutaneous nerves also induces local blanching. Hence in Mustelus active blanching is nervous though favored by the absence of intermedin. In this fish then darkening is due to intermedin and blanching is the result of the action of concentrating nerves (Parker and Porter). Blanching in Mustelus, suspected of being caused by vasomotor changes (Hogben, Wykes), has been shown to be independent of circulatory disturbances (Parker). Another chromatic vertebrate which appears to have a combined system partly humoral and partly nervous is the horned toad (Phrynosoma), a lizard of the North American deserts. As a matter of fact the chromatophoral system in this creature resembles in a striking way that in Mustelus, for the darkening of Phrynosoma is due to intermedin and its blanching is the result of the action of concentrating nerves (Redfield, Parker).

The third example of color change to which I wish to call your attention is that of the catfish Ameiurus. This fish has a melanophore system which enables it to range from a very pale greenish yellow to coal-black. Its dark phase is dependent chiefly upon intermedin from its pituitary gland. If in a pale fish one of its radiating caudal nerves is cut, a dark band corresponding to the area of distribution of the nerve will form. This type of response has long been known in other chromatic vertebrates and has been commonly attributed to paralysis. If, however, a pale fish in which such a caudal band has been formed is kept in a white-walled illuminated aquarium, the band will blanch in a few days. If now the nerve is cut a second time and at a point a little distal to the first cut, a second band will form and remain conspicuous for a day or two. The formation of this second band shows that the production of such bands can not be due to paralysis, for the nerve to form this renewed band must be still active. The nerve is without doubt reactivated by the second cut, and the particular fibers thus brought into action are dispersing fibers. This test and others like it have led to the conclusion that Ameiurus darkens not only through the action of intermedin but also by means of dispersing nerves. Ameiurus blanches in consequence of concentrating nerves whose action in this and many other fishes has long since been demonstrated by electric stimulation. Thus the melanophores of Ameiurus have a double innervation, one set of nerve-fibers controlling the dispersion of pigment and the other the concentration of this material. Such a double innervation is consistent with the histological discoveries of Eberth, Ballowitz, and Eberth and Bunge all of whom have shown that two or more nerve-fibers may reach the same melanophore. The double innervation of teleost melanophores has also been demonstrated in studies of partly innervated areas and in the regeneration of melanophore nerves (Mills, Parker and Porter, Abramowitz). The catfish then represents what may be regarded as an extreme instance in chromatic complication in that its color system involves concentrating and dispersing nerve-fibers and intermedin. This complex system has been shown to occur in a number of other teleosts, and it is probably present in the African chameleon. Thus from the frog with its possible single chromatic humor, intermedin, one may pass to mixed chromatic systems with one set of nerve-fibers and intermedin, as in Mustelus, or to those with two sets of fibers and intermedin, as in Ameiurus. No chromatic vertebrate is known that does not possess intermedin as a darkening agent, though in some, as for instance Fundulus, this agent may be very subordinate. Mixed systems, partly humoral and partly nervous, occur among fishes and lizards. Apparently there is no instance in the vertebrates of a purely nervous chromatic system.

What are the details of melanophore activation? Of the agents to this end intermedin is the most obvious and universal in the vertebrates. Under appropriate environmental conditions this substance is discharged from the pituitary gland of a given chromatic animal into its blood by which the intermedin is carried over the animal's body to its melanophores. These cells are thereby excited to disperse their pigment. Thus intermedin appears to act directly and immediately on the color cells themselves. The way in which the chromatic nerves, dispersing and concentrating, influence melanophores can be surmised best by studying the blanching of dark bands in the tails of such forms as the killifish and the catfish.

A dark band produced in the tail of a pale killifish or a pale catfish by cutting a bundle of chromatic nerve-fibers will blanch in such a fish on a white background in one or more days. Such a band does not blanch uniformly but begins to fade first on its edges leaving its axis dark. Finally the dark axis fades completely. In the killifish bands one millimeter wide blanch on the average in about 30 hours, those two millimeters wide, in some 78 hours (Parker). In a catfish a band one ray in width becomes pale in 2 days, one two rays wide in 5 days and one three rays wide in 10 days (Abramowitz). These peculiarities show that the fading band is not influenced uniformly as, for instance, from below by the blood and lymph, but that it is changed by something that affects it laterally. Either a substance escapes from the sides of the band and by its absence allows the lateral melanophores to concentrate their pigment or an external agent makes its way from the surrounding tissue into the edges of the band and thus induces pigment concentration. Matsushita has performed an experiment which shows that the second of these alternatives is the correct one. If a new caudal band is cut in a pale fish and in such a position that on one of its sides there is a fully blanched denervated band and on the other the normal innervated pale area of the tail, its method of fading will be significant. Matsushita showed that it fades only on that side which is next the innervated pale area and not on the opposite denervated side. Its blanching is therefore not due to the loss of a darkening substance, for this could escape equally from either side. It must result from the invasion of a blanching agent whose source is the innervated pale portion of the tail. In a somewhat similar way it has been shown that a denervated pale band will darken over only that portion of its extent which is flanked by two newly formed dark bands of half the length of the pale band and not over that part not so flanked (Parker). Thus there is good reason to conclude that pale innervated regions contain an active concentrating substance and that dark nervously active regions contain an equally effective dispersing substance. Both these substances may pass by slow degrees from their regions of origin into adjacent regions. Since the regions of origin of the concentrating substance are the regions of active concentrating nerve-fibers and the regions of origin of the dispersing substance are the regions of activated dispersing fibers, it is natural to conclude that these substances are the products of their respective sets of nerve-fibers each in its particular way activating the melanophores concerned.

It has already been pointed out that these substances do not act as though they were dissolved in blood or in lymph. They are much too circumscribed in their effects and much too slow in their spread to be in aqueous solution. The only other constituents of the tissues in which they are likely to be dissolved are the lipoids, and it is probable that these activating substances are carried in such fatty materials and that they migrate by diffusing slowly through such materials. This view is supported by the fact that an Italian olive-oil extract of a dark catfish skin when injected as a coarse emulsion under the skin of a pale catfish will cause the formation of areas of dark color. These dark areas can not be produced by injections of pure olive oil or by oil extracts of tissues of other than dark skin. They are therefore believed to be due to some substance dissolved from the dark skin by the oil. Such oil-soluble materials have been called lipohumors in contrast to hydrohumors like, for instance, intermedin which, as already indicated, act in aqueous solution on melanophores (Parker).

Although the dispersing and the concentrating lipohumors of the catfish give evidence of being normally dissolved in the lipoid constituents of its skin, these humors appear to be also soluble in water. Dissolved in this medium they may be tested for their chemical characteristics. Aqueous extracts of the dark skins of catfishes when appropriately prepared and applied to the muscle of the leech give every evidence of containing acetylcholine. This substance moreover when injected into a catfish previously treated with eserine will darken the fish. It is therefore probable that the darkening lipohumor of the catfish is acetylcholine (Chin; Chang, Haieh, and Lu; Parker). This substance has long been known to be a most remarkably efficient agent in many organic operations, and this peculiarity appears in the darkening of the catfish, for the skin of this creature is of a deep hue when it contains one part by weight of acetylcholine to about 13,000,000 parts of skin.

If it is probable that the dispersing nerve-fibers of the catfish act on melanophores through the neurohumor acetylcholine, what can be said of the concentrating fibers? Here aqueous solutions may also be used in tests. When such solutions properly prepared are applied to the frog's heart they are found to give evidence of containing adrenaline (Parker). As has long been known this substance when injected directly into a dark catfish will cause it to blanch. Hence there is reason to believe that just as the dispersing nerve-fibers probably act on melanophores through the production of acetylcholine, so the concentrating fibers affect melanophores by the discharge of adrenaline. This substance, though a highly efficient agent in the control of organic activities, is by no means so remarkable in this respect as acetylcholine. A catfish will blanch when its skin contains one part by weight of adrenaline in 350,000 parts of skin. Thus in the catfish the concentrating fibers appear to produce adrenaline and may be classed therefore as adrenergic; and the dispersing fibers acetylcholine and hence classed as cholinergic.

The nervous component of the melanophore system in the catfish is therefore organized upon the plan of opposing sets of nerve-fibers with their appropriate neurohumors as occurs in other parts of the autonomic system. But the melanophore system in this fish has in addition to these two neurohumors a third one, intermedin, which, so far as is known, has not been identified in other parts of the nervous organization of vertebrates. Nor is this the limit, for the chromatophore system in other fishes includes in addition to melanophores other types of color cells such as xanthophores, erythrophores, leucophores and the like, none of whose activities has been so fully analyzed as those of the melanophores, yet whose functional performances are such as to imply the presence of other and different neurohumors. Thus the conditions in chromatic vertebrates give promise of a considerable array of these substances still to be discovered.

But more important than a promised increase in the number of neurohumors is the peculiar behavior of the two nervous components in the catfish to their organic environment. Acetylcholine when introduced in aqueous solution into the body of a catfish is quickly destroyed by the cholinesterase of the animal's tissues. If, however, it is introduced dissolved in oil, it will remain effective for days. Apparently it is protected from such agents as cholinesterase by being in solution in the oil and it only slowly and gradually diffuses out from this medium into the adjacent aqueous environment. Here it may act momentarily on the melanophores before it suffers destruction. Thus oily deposits in general, including the cell lipoids, may serve as protective reservoirs for acetylcholine and in consequence greatly lengthen the period of this agent's activity. In this way the darkening action of acetylcholine may be greatly prolonged.

The same seems to be true of adrenaline. Although this substance is not rendered ineffective in the fish's body with any approximation to the rapidity with which acetylcholine is, it is nevertheless none too stable and in oily mixtures its activity far outlasts in point of time that which it shows in purely aqueous solution. This feature of neurohumoral activity is not without general biological significance, for it points to lipoids and other like substances as possibly important protective repositories for susceptible agents whose existence in aqueous solution in the organism might be very limited and precarious.

From this brief survey of the means of activation of a limited group of chromatophores it must be evident that neurohumors not only exist in animals, but that they exist in reasonable variety. They may be defined as hormones produced by nervous tissue or by glands appended to that tissue and concerned with the activation or inhibition of other nervous tissue or its effectors. That they occur in many parts of the nervous system is beyond doubt; that they serve in certain regions as means of synaptic transmission is highly probable; but that they are the exclusive means of such transmission can not at present be affirmed. This uncertainty leaves open still the possibility of electrical transmission in certain places. It is, however, conceivable that even here neurohumoral transmission may also occur and that in such instances these two supposedly different types of transmission may be merely two aspects of the same operation (Forbes). In conclusion it may be said that neurohumoral transmission offers a natural explanation for synaptic polarization, for if one side of the synapse is secretory and the other receptive, transmission would naturally be in one direction only. Moreover such a double operation might well be expected to involve synaptic delay not to be looked for as a necessary step in electric transmission. Because of these features the neurohumoral rather than the electric conception of transmission at the synapse has been gaining ground and the general conception that complex nervous activity involves a myriad of momentary neurohumoral discharges and receptions is coming to be a significant picture in the minds of modern neurophysiologists (Dale).