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NEUROHUMORS: NOVEL AGENTS IN THE ACTION OF THE NERVOUS SYSTEM[1]

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THE enunciation of the neurone theory by Waldeyer in 1891 was a fitting culmination of the neurological work of the nineteenth century. By means of it many detailed questions on the structure and the function of the nervous elements found satisfactory and final answers, but as a result of it there also arose a host of new and perplexing problems, many of which are still unsolved. Prominent among these is that concerning the relations of nerve-cells or neurones not only among themselves but also between them and the cells of receptor and effector organs.

Embryonic nerve-cells or neuroblasts are at the outset reasonably separate and independent entities without special functional interdependence. As they grow and differentiate they come to form systems of conducting pathways by which one remote part of the body is brought into nervous connection with another. How neurones are related in such conducting systems has been a matter of dispute. Some histologists have claimed that the processes of one neurone fuse with those of the next and thus establish possibilities of nervous conduction; others have declared that such processes are only in contact one with another. The importance of this question disappeared, however, when it was found that degenerative changes started in one neurone never pass over the assumed boundary into the next neurone and that nerve impulses, which may course in either direction up or down a neurone, are limited to one direction in passing from neurone to neurone. Thus, whether neurone tips are fused with each other or are merely in contact, their region of joining, the so-called synapse, must be a differentiated area polarized as to its direction of transmis-

[1] An address given at the annual meeting of the Worcester Chapter of the Sigma Xi, on November 5, 1934.

sion. Thus in a way the old and rather discredited "law of forward conduction" receives a certain restricted justification.

The capacity of the synapse to limit the direction of transmission has often been compared to the action of a valve. But this is obviously a figurative statement, for the nerve impulse is not a gush of fluid through a tube whose direction of transmission is controlled by valvular devices. What, may we ask, is it that passes over the synapse? Two possible answers to this question have been put very clearly by Gerard, who has stated that either the same kind of ion migration and chemical change which represents the impulse itself passes over the synapse from neurone to neurone or the terminals of the discharging neurone act as miniature glands and, when stimulated, produce some chemical which is able to excite the tips of the next neurone and thereby initiate another impulse. Since the first of these hypotheses offers no explanation for synaptic polarization, while the second does, modern opinion has drifted consistently toward the latter. This view implies a chemical interpretation of the interaction of nervous elements, a view which in a certain measure was long ago advocated for sense-cells and their conducting elements by Botezat. It is to be met with continually in the writings of the late Ramón-y-Cajal and has been suggested for central organs by Sir Charles Sherrington and his associates. The chemical substance produced in this assumed activity, whereby one neurone may excite a neighboring neurone or other appended cell, has been called a neurohumor, to use a term introduced some years ago by Henri Fredericq. Such substances include in my opinion not only materials like acetyl choline as recently discussed from this standpoint by Sir Henry Dale and his fellow workers and Cannon's sympathin, but also adrenalin and those products of the pituitary gland which are known to activate animal effectors.

Neurohumors may act over distances of microscopic if not ultramicroscopic proportions or over large ranges in the animal body. Such substances, irrespective of their extent of spread, are in principle nervous activators and their grouping under one head, namely, that of neurohumors, is fully justifiable. Some students of this subject would class all neurohumors as hormones; others would include under this heading only such as act over long distances, but this does not seem to me to be a matter of serious import. Neurohumors really act as hormones over shorter or longer ranges. The precise problem that we have to face is not the classification of neurohumors, but the extent to which these substances actually exist. To that question we may now address ourselves, and in approaching it I shall deal chiefly with the nervous control of the melanophores in fishes.

In 1876 Pouchet showed that if the integumentary nerves of a turbot are cut, the denervated area of skin thus produced becomes dark through the dispersion of the melanin granules contained within its melanophores. The nerve-fibers concerned in this operation were shown to be autonomic in origin. Their great abundance and button-like terminals were demonstrated in a number of fishes by Ballowitz in 1893. As a result of these discoveries the nervous control of melanophores in fishes was accepted by practically all workers in this field, a conclusion amply supported by the later exhaustive investigations of von Frisch. The neurohumoral interpretation of these results is to the effect that the nerve-terminals applied to the chromatophores do not excite these cells directly, as is generally believed, but that these endings secrete a neurohumor which on reaching the color-cells induces them to respond in an appropriate way. Is there any evidence that such neurohumors play a real part in the color changes of fishes? A test of this question has been attempted on the common killifish, *Fundulus heteroclitus,* which has well-marked dark and light phases.

If a small transverse cut about a millimeter in length is made through a single fin-ray near the root of the tail of a light Fundulus, small bundles of radial nerve-fibers going to a restricted part of the tail will be severed and the denervated area thus produced will become evident as a dark, radiating band extending from the cut to the posterior margin of the tail. Such a band will begin to appear in about half a minute after the cut has been made and will grow in intensity for a short time, after which it may remain visible for several days. Under the microscope the band can be seen to be made up of melanophores whose pigment is fully dispersed, a condition in strong contrast with that of the color-cells in the rest of the tail where the melanin is densely concentrated near the cell centers.

Such a dark denervated band will maintain itself for as much as several days, even though the fish on which it has been formed is kept in a white-walled, illuminated aquarium, a condition under which the light coloration of the fish as a whole is retained. Gradually, however, the band begins to blanch and sooner or later it disappears by taking on the light tint of the fish. Bands of this kind may be called primary bands.

If such a light fish with a completely or nearly completely blanched primary band is put in a black-walled, illuminated aquarium, the fish, with the exception of the band, turns dark in less than two hours. The band, though denervated, then also darkens but only gradually and finally in a little less than a day it becomes as dark as the rest of the fish. The converse change follows a corresponding course. When a dark fish with a denervated band in its tail is put into a white-walled aquarium, the fish as a whole blanches in rather less than five hours and the band in a little over a day. Bands either light or dark that are produced after the initial or primary band has disappeared may be called secondary bands and such bands change with the changes in the surroundings as the body of the fish does but with a very considerable lag.

When the fading of a primary band is followed in detail, it is seen that the band does not blanch uniformly and as a whole, but it begins to disappear first on its edges, as pointed out several years ago by Mills, and this process gradually spreads towards its axis, which is the last part to fade. The disappearance and reappearance of secondary bands also take place by lateral disintegration. According to the neurohumoral hypothesis, this process is to be understood as a response on the part of the denervated band to materials produced in the adjacent innervated portion of the tail. These materials make their way gradually from the regions of their origin into the band and thus effect a change in the melanophores there corresponding to that seen in the innervated area. In fact, it seems to me very difficult to explain these changes in any other way. It is well known, for instance, that these changes can have nothing to do with the possible degeneration or regeneration of the local nerve-fibers, for it has been shown that these fibers do not degenerate till some twelve days after they have been cut and that they regenerate in only about twenty to twenty-five days after this operation. The disappearance and reappearance of the bands, as already described, may be excited any time after the blanching of the primary band, that is, after two or three days following the exciting cut. That the nerve-fibers in a blanched primary band are still fully active can be shown by recutting such a band in a region slightly distal to the initial cut. By this means the band may be fully revived from the new cut to the free edge of the tail, showing that in its blanched condition the nerves in the band have gone into a temporarily quiescent state and are in no sense degenerated.

The primary band is apparently due to nerve impulses which for a time after the initiating cut has been made emanate from that region and excite the more distally located melanophores to disperse their pigment. If such an assumption is correct, it ought to be possible by an appropriate block to intercept such impulses and thereby obliterate the band. The most appropriate means of doing this is cold. If a capillary glass tube carrying dilute alcohol chilled to a degree well below 0° C. is applied to a fairly mature primary band, the distal part of the band that is separated from the cut by the tube soon blanches. This response justified the view that the band is dependent upon a flow of impulses from the region of the initiating cut.

How these impulses are produced is not easily stated. Since in the normal locomotion of the fish the tail is more or less continuously moved from side to side, it might be supposed that the friction thus generated in the cut would be the means of exciting impulses. If, however, in place of the usual small transverse incision in the tail, a square window is cut therein whereby the cut faces of the wound are no longer capable of rubbing one against the other, the band will nevertheless appear. It must, therefore, be admitted that the nerve impulses that call forth the band are not the simple result of the rubbing of the faces of the wound. How these impulses originate can not be stated. Probably they are dependent upon some more subtle form of stimulation in the cut, for it seems clear that the primary band is due to a flow of impulses from the cut region to the more distal melanophores. With the subsidence of this excitation the primary band gradually disappears, a step much in advance of the degeneration of the nerve-fibers in the region concerned.

After the disappearance of the primary dark band the revival of bands which resemble but lag behind the bodily color-changes have been ascribed to neurohumors and presumably to two sets of these substances one exciting a concentration of melanin in the containing cells and the other its dispersion. This interpretation suggests double innervation for these cells, a belief that is substantiated by the fact that when the exact areas of a given dark band and of its light equivalent are accurately compared, they are found not to agree precisely (Mills). This state of affairs is difficult to understand, except on the assumption of a double set of nerve-fibers, one for pigment concentration and the other for its dispersion.

If two sets of nerve-fibers are present for the melanophores in Fundulus, two sets of neurohumors are to be expected. Evidence in favor of the duplicity of these agents is afforded by experiments with flanking dark bands. Such experiments are best seen in the tails of the catfish Ameiurus, though they can be demonstrated in the tails of other fishes such as Fundulus. If two short, initial, dark bands are produced in the tail of a light catfish and in such positions that an intervening band of innervated tissue is left between them, the flanking bands of course darken; the area between them, however, remains light. If now the same test is tried but with a difference that the intervening band is a denervated blanched one and the dark flanking bands are short ones and of such a length as to abut only the distal half of the blanched band, this band in a short time shows a remarkable state. Its proximal half, that which adjoins the light area of the tail, remains light, while its distal half, flanked by the newly formed dark bands, becomes conspicuously dark. From these experiments two conclusions can be drawn: first, that from the dark flanking bands something makes its way into the denervated light band and darkens it, a dispersing neurohumor; and, second, that an innervated light band can resist this darkening by something produced by its nerve-fibers, a concentrating neurohumor. It seems highly probable, therefore, that there are not only concentrating and dispersing nerve-fibers but also corresponding neurohumors.

The spread of these neurohumors over the millimeter or two of integument which may constitute the width of a band is from the side of the band inward toward its axis. This spread takes place at a very slow rate, the whole operation often requiring as much as a day. This slow axial spread makes it very improbable that the transfer of the neurohumors is by means of the blood and lymph of the given region. That blood and lymph are not concerned in this operation is shown in at least two ways. If adrenalin is injected into a Fundulus with a dark caudal band, this band disappears within less than a quarter of an hour and disappears as a whole, not by disintegration from the sides. This type of disappearance is what would be expected from a neurohumor carried in the blood, for the whole undersurface of the band is open to approach from the circulatory system. Further, as Matthews has recently shown, the blood from a dark Fundulus does not produce a dark spot when injected into a light Fundulus and *vice versa*. From these two standpoints it, therefore, seems clear that if neurohumors are produced by the melanophore nerves in Fundulus, these are not transmitted to the color-cells by the blood. They are probably not water-soluble. That they do make their way across the millimeter or more of denervated skin in the band can not be doubted. Hence it is concluded that they must be carried by some other solvent than water. I have expressed the opinion that they are soluble in oil and that they are transmitted from cell to cell by the fatty or lipoid constituents of these bodies. Such a means of transmission is quite consistent with the facts that have been learned concerning the appearance and disappearance of caudal bands, particularly the lateral decay of these bands and their notable lag behind the color changes of the fish as a whole.

A conclusion such as that just arrived at naturally raises the question of evidence for or against oil-soluble neurohumors. An attempt to ascertain whether there are such neurohumors has been made on the common dogfish, *Mustelus canis*. This fish, like Fundulus, has two well-marked color phases, one dark and the other light. As might be expected, the dark phase results from the dispersion of melanin in the dermal melanophores and the light one from its concentration. As Lundstrom and Bard showed in 1932, when the pituitary gland of a dogfish is removed, the fish becomes permanently light-tinted. On injecting a water extract of the gland into such a light fish the dark phase is temporarily reassumed. Moreover, the injection of defibrinated blood from a dark fish into a light one produces a temporary dark spot about the region of injection. These facts led Lundstrom and Bard to conclude that the dark phase of Mustelus is due to a substance produced in the pituitary gland and carried from that gland in the blood of the animal to its dermal melanophores.

Two years later Parker and Porter showed that when the integumentary nerves of a dogfish are cut, the denervated area thus produced becomes very light, even lighter in tint than that of the ordinary light dogfish. It, therefore, appears that in the dogfish, although the dark phase is produced by a dispersing neurohumor carried in the blood, the light phase is a nerve response of strictly local occurrence. The question naturally arises, Is there a neurohumor associated with this light phase? As has already been indicated, when the defibrinated blood of a light dogfish is injected into a dark one, no color change can be detected. Hence if the light phase is induced by a neurohumor, the neurohumor involved, since it is not carried in the blood, is probably not soluble in water. Is it soluble in oil? To test this question the fins of a light dogfish, the most responsive parts of its body, were reduced to a pulp by grinding them in a pulping machine. This pulp was then extracted with pure Italian olive oil. The oily residue thus obtained was injected with proper precautions into a dark dogfish, which in course of time showed a striking light spot near the region of injection. Under the microscope the melanophore pigment in this spot was seen to be concentrated in the color-cells, a condition that could be temporarily overcome by the injection of pituitrin into the animal. Light spots of the kind described did not result from simple oil injections. An ether extract of the fins also induced the formation of light spots in the fish. Extracts from fresh fins or from fins that had been dried for over a day at 110° C. were equally effective. These observations support the conclusion that in Mustelus beside the water-soluble pituitary neurohumor by which the melanophores are induced to disperse their melanin and thereby darken the fish, there is also an oil-soluble neurohumor that is equally effective in bringing about pigment concentration. This at least seems reasonable in view of what has already been described for the concentrating neurohumor of Fundulus. These studies on the so-called nervous control of the melanophores of fishes point with great certainty to neurohumors as the agents really concerned and suggest the probability that there are two classes of such agents, one of which consists of materials, like pituitrin, soluble in water and hence transportable by the blood, hydroneurohumors, and the other soluble in oil and hence transmissible through the fatty or lipoid constituent of the tissues, liponeurohumors.

Although the instances here discussed are taken from only a single group of effectors, chromatophores, and their nervous connections, it is possible that reactions of this kind extend throughout the whole of the nervous organization of animals and that the relation of receptor cells to neurones, of one neurone to another, as well as of neurones to effectors, may be based upon the same principle that appears to apply to chromatophores. This in fact is the neurohumoral hypothesis, a view which in its essence has been expressed already by a number of workers and which under the general caption of the chemical interrelation of nervous elements has permeated the thinking of not a few of the neurologists of to-day.

THE UNSOLVED PROBLEMS OF LEPROSY[1]

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THE earliest medical records from Egypt and India are said to include descriptions of clinical leprosy.[2] Although the somewhat indeterminate description of cutaneous ills summarized in the term "zaraath" in the Old Testament may well have included several separate entities there is every reason to believe that it was incidentally descriptive of the disease now known as leprosy. The horror, fear and pity which leprosy in its exaggerated forms have always excited led to early attempts at segregation of its sufferers in many places. At all events the modern clinical description of leprosy dates from the work of Danielsenn and Boeck in 1847. It seems certain that Hansen in 1868, and even before he had anilin dyes to use, actually described with fidelity the massive aggregates or globi of the leprosy bacillus which we now recognize as a constant feature in the cutaneous form of the disease. In spite of these two relatively early evidences of objective certainty, the history of leprosy as a process remains in many of its significant phases as baffling to-day as it was a century ago. It may well take all the short time placed at my disposal to list with anything like sequential probability what the outstanding and unsolved problems of leprosy are, but this, at least, I shall attempt to do, with the further hope that I may emphasize two of the main junction points in the historical pathway which this brief survey covers.

It is still uncertain precisely what effect the segregation of lepers has had in suppressing the disease. Such isolation can never be completely carried out, particularly under the conditions of living in those countries where it is most prevalent, and a belief in its effectiveness is based largely on the disappearance of leprosy from Continental Europe in the Middle Ages, although certain other factors, such as other decimating epidemics, changes in dietary and even of climate, may well be involved. At all events the disease still remains in precisely those localities of the world where it was first described, in Africa, in the Orient and in the West Indies. Until we know more fully the precise epidemiology of leprosy no process of segregation could be expected to be efficient.

There is no question as to the great prevalence of leprosy in the early years of life, and the modern trend of thought goes farther in believing that infection takes place, at least in the majority of instances, in these very same early years, irrespective of the precise time of its clinical detection. We would not go so far as does Manaling, who, in view of the slow development of the disease and its frequent spontaneous cure in children before the complete evolution of the disease, would deny the possibility of its inception in adult life. In fact, there are recent well-controlled instances of accidental and experimental infection which render it probable that natural adult infection normally, although perhaps rarely, occurs.[3]

Far too little attempt, it would seem to us, has been made to ascertain the possibility of eliminating leprosy by immediate segregation at birth. Most of the references on the effect of removing the offspring of leper parents are misquoted and deal rather with separation at varying periods after birth than at the moment of delivery. The extraordinarily important results reported by Hasseltine in Honolulu have not, so far as we are able to determine from the literature, or on direct inquiry from those most concerned, been followed up. It will be recalled that Hasseltine found

[1] Address delivered before Section N—Medical Sciences, American Association for the Advancement of Science, Pittsburgh, December 31, 1934.

[2] Rogers and Muir.

[3] deLangen, Marchoux.