

# THE BIOLOGICAL BULLETIN

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## THE CHROMATOPHORES OF THE CEPHALOPODS<sup>1</sup>

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*Ladies and Gentlemen:* It is with the greatest pleasure that I am speaking this evening, as a member of the Stazione Zoologica of Naples, here in the auditorium of the Woods Hole Laboratory. I am glad to bring to every one of you the best greetings of us all, and to assure you that, when three years hence you come to Italy to the next Physiological Congress, and next year when some of you go to Padua for the Zoölogical Congress, we hope you will gather in the Stazione Zoologica in as great a number as you have gathered here. I do not know if we shall be able to arrange things as well as you did here, but I can promise that our welcome will be as hearty as yours and that we shall do our best to this purpose.

Ladies and gentlemen, it is impossible to look at the beautiful play of the chromatophores of the cephalopods—the object of our lecture this evening—without being filled with wonder at the complication and the perfection of the play itself. Old Aristotle was the first to study these animals with the eyes of the scientist, and he was also the first to connect some of the different appearances shown by the animals with various conditions of their minds: that is, fear and anger, and so on. He was also the first to suggest that these animals were able to change their colour according to the colour of the environment, giving, in this way, one of the first instances of this fundamental law of living beings, namely, mimicry. It may be of interest to remember that this opinion of his has been confirmed in a most brilliant way quite recently by the careful experiments of some German scientists (Kuehn and Heberdey, 1929).

As in many other instances, Aristotle was more than twenty centuries ahead of his time. It was not until the beginning of the nine-

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teenth century that people began to study again the characters and the mechanism of the colour-changes in cephalopods. Opinions on the nature of colour-changes differed very widely at that time, and some of them may be quoted as instances of the ways in which, if not controlled by observation and experiment, scientific thought can be misled. It was believed at first that the colour was produced by some coloured liquid; but very soon it was discovered that the coloured spots were stable and that they did not change their place, but only their form. They were constant formations, differentiated from some part of the skin. The name of Sangiovanni should be remembered at this point, because he was the first to recognize (1819) that these colour-changes were the effect of some special little organs. To these he gave the name *chromophore*, which has ever since clung to them, although in a slightly modified form, *chromatophores*, that is to say, colour-bearers.

After this first fundamental discovery, which laid the foundation of all subsequent morphological investigations, research-work developed along different lines. It would take us too long to follow closely their development through the nineteenth century to the present time. Out of this work two points have become clear, which it would be well to emphasize now in order to get a better understanding of what we are going to say later on. The first is that the expanded condition of the chromatophores is the active one, just the opposite of what we are accustomed to find in the vertebrates, for example, in the frog. This fact may give rise to several misunderstandings and misinterpretations, because of the opposite meaning that the same word is bound to have when applied to the chromatophores or to the muscular fibers. In order to avoid these misunderstandings, we shall, once for all, decide, after Steinach, to speak of expansion and retraction of the chromatophores, to which correspond respectively the contraction and relaxation of the muscular fibers. The second point of importance was the definite recognition (by Steinach, 1901) of the muscular nature of the radial fibers of the chromatophores, a fact which was established after manifold and lengthy debates, in spite of the strenuous opposition of some investigators. These two facts are undoubtedly connected, and they both bring us to consider more closely the morphology of the chromatophores.

In the frog, as well as in other vertebrates and in the crustaceans, chromatophores are single cells, whose contractile properties resemble more nearly amoeboid contractility than that of even slowly contracting muscular tissue. In the contracted (or "active") condition, the chromatophore looks like a point or like a little sphere and resembles

an encysted amœba. From this extreme contracted or active condition all stages up to extreme expansion can be observed. Little by little the chromatophore cell gives out prolongations—we may as well call them pseudopodia—which expand more and more. At the end they are so widely expanded as to penetrate between the neighbouring chromatophores and to form in this way an almost continuous layer of coloured cells.

The chromatophores of the frog, and of vertebrates in general, are therefore to be considered as a very peculiar sort of formation; together with the leucocytes, the only representatives, in the realm of more highly organized beings, of cells endowed with this fundamental property of protoplasm, *i.e.*, contractility, in its most elementary form.

The case of the chromatophores of the cephalopods is completely different. It cannot be our task to discuss the question of their descent from one or more cells. What matters for us is that in their definitive condition they are formed by a sort of little sack and by a series of radially distributed fibers. The sack is filled with pigment granules and its walls possess elastic properties, which are the primary cause for the rounded, or at least elliptical, shape of the chromatophores in the resting condition. The fibers, on the other hand, have their movable end on the walls of the sack, their fixed one somewhere in the skin. The movable ends on the wall are at regular intervals, arranged in such a way that the whole looks like a radial figure. The fibers, as mentioned above, are muscular in nature, and therefore, every time they are reached by a stimulation and contract, the walls of the sack become expanded and remain expanded as long as the contraction of the fibers lasts. As soon as the fibers relax, the wall of the sack, because of its elastic properties, goes back to its resting, *i.e.*, retracted condition.

In some instances it can be noticed that under certain natural or experimental conditions the chromatophores may retract still more. To this day it cannot be decided whether this depends upon the inhibition, in these cases, of a sort of residual tonus of the fibers, which generally hinders the full action of the elasticity of the wall, or upon the existence of some sort of device for the purpose of active retraction. This last has been postulated by some authors, but, although it cannot be definitely denied, no satisfactory evidence of anything likely to produce such an effect has been reported so far.

It should be clear from this description that the expansion of the chromatophores is produced by the combined pull of many (sometimes 20–25) fibers at the same time. Every one of these individual

fibers pulls in its own direction, but, as they are radially distributed, it follows that the chromatophore expands as a whole and almost maintains its form, which may now be described as a more or less regular polygon, whose rather rounded edges correspond to the points where each fiber is attached.

As mentioned above, under "normal" conditions all fibers of a given chromatophore pull at the same time, and as they have no direct connection whatever between themselves (excepting through the wall of the chromatophore itself), this fact, which is one of very perfect coördination, points to some kind of external control. As a matter of fact, under normal conditions the chromatophores are strictly and continuously controlled by the central nervous system. As soon as the connection with this is severed, the chromatophores are completely paralyzed and, for some time at least, they remain in a condition of complete rest unless artificially stimulated by way of the peripheral nerves. The demonstration of this fact, for which we are indebted to Leon Fredericq (1878), is a very easy one. If, in any species of cephalopods, the so-called mantle-connective is cut, the chromatophores of the corresponding half of the body, as well as the mantle-muscle itself, are instantly and forever paralyzed. However excited the animal may be, however beautiful and intense the play of its chromatophores on all other parts of the skin, those on the half whose nerve has been cut will never again reproduce the same phenomena. The play of the chromatophores, the normal one at least, is produced by impulses coming from the central nervous system: the mantle-ganglion, which, in the experiment just described, is still connected with the chromatophores, is completely unable to produce any spontaneous or reflex expansion. It seems that the ganglion has nothing to do with the chromatophores of the mantle, and that the nerves to these last go directly, without any stop, from the central ganglia to the peripheral plexi.

Now we begin to know something about the forces controlling the chromatophores. The next problem to consider is that of the afferent paths of the reflex arc whose efferent paths are represented by the nerves going to the chromatophores: that means that it is necessary to decide to which stimuli the centers for the chromatophores respond.

We have related above the answer which Aristotle gave to this question. He admitted, rather briefly we must say, that anger and fear and similar feelings were the primary cause for the colour-changes of the sea-devils and of the squids. We will not deny this opinion; much more, we shall support it in a rather unexpected way. A physiologist, however, cannot remain satisfied with these rather vague

expressions and is bound to analyze these phenomena somewhat more deeply. The analysis has been carried on by different authors and the importance of several sorts of stimuli has been recognized.

First of all, it is through the eyes that the animal receives the impressions that produce the changes in its colour. Anybody may notice that the colour of the animal changes whenever it sees any object which attracts its attention. That is the phenomenon noticed by Aristotle. It is possible, however, to have some more exact idea of the importance of the visual impressions for the colour-changes by determining how far the animal still maintains its capacity for changing its colour when the function of one, or both eyes is excluded. The play of the chromatophores is not abolished in either case, but the capacity which the normal animal has for changing its colour with the colour of the environment is lost. For this sort of adaptation at least, visual impressions are of the first importance; but, in contrast to what happens in some fishes, they are not the only ones controlling the expansion of the chromatophores. It is interesting to remember that when only one eye is put out of function, the activities of the chromatophores of the corresponding half are somewhat diminished. They answer to all stimuli as well as those of the other half, but somewhat more slowly, and their expansion is often less complete. This phenomenon shows that although the stimuli reaching each eye act on all coloration centers of the central nervous system, they affect primarily those of the corresponding half, and their action on those of the opposite half is somewhat more indirect.

The animal deprived of its visual impressions still maintains its capacity for changing its colour, although this function is somewhat impaired. It was the merit of Steinach (1901) to demonstrate that another set of impressions controlling the play of the chromatophores is that issuing from the suckers. There is no doubt that the importance of these excitations for the play of the chromatophores was somewhat exaggerated at first, but it is impossible to deny that, if all suckers are extirpated, the "tonus" of the chromatophores is much diminished, and their movements much less frequent and vigorous. The animal, deprived of its suckers, or of both suckers and eyes, has not yet lost its colour-controlling capacity, although this is much impaired. It still answers with a change of colour, just as does the normal animal, to many kinds of stimuli: for instance, to vigorous stirring of the water (which probably amounts to both an excitation of the sense terminations in the skin and of the statocysts), to pricking or pinching the skin, placing on its back, and so on.

The phenomena we have just described show that there are not

specific stimuli affecting the centers of the chromatophores. The latter may answer a variety of excitations, none of which can be described as alone responsible for the changes of colour, although any one of them can, at any given moment, induce them. The center, or better, as we shall see later, the centers for coloration are the effector centers for many, we may as well say for all, sorts of excitations, every one of which may affect them in turn, or together with others. The excitations from the eyes and from the suckers assume a somewhat more prominent place. This is only because, more often than the others, they affect not only the momentary conditions of the chromatophores, but also their "tonus," that is to say, the condition of greater or lesser expansion which prevails for some time and on which the momentary changes superpose.

There is a third group of excitations of which little or no notice has been taken until now, and which, without any doubt, along with those from the eyes and suckers, plays a very important part in determining the "tonus" of the chromatophores in the normal animal (Sereni, 1928a). It is easy to notice that the ventral side, both of the body and the tentacles, is generally less coloured than the dorsal one. This depends partly upon a morphological difference, namely, a somewhat different distribution of the chromatophores. A closer observation, however, shows that the chromatophores of the ventral side are much less expanded. This fact corresponds to a very general phenomenon among sea animals, whose ventral side is very often less coloured than the dorsal one. This may be considered as a sort of protective coloration because, when seen from below, the sea surface has a whitish colour. However this may be, there is a more immediate cause for it. In the cephalopods the difference in the grade of expansion between the chromatophores of the ventral side and those of the dorsal side is specially marked when the animal lies on the ground or is fixed to the walls of the basin. In such cases, as anybody can see, there is no danger of the animal being attacked from below and any sort of protective device is excluded. It seems much more likely that in this case we have to do with a sort of postural reflex of the chromatophores. As a matter of fact, if, in the normal animal by some special trick, we succeed in placing the animal on its back or on one of its lateral sides, we find that the chromatophores are less expanded upon whichever side the animal is lying. This is more easily done with animals deprived of their eyes and of their suckers. If we turn the animal on another side, the change in the grade of expansion of the chromatophores is immediate. The mechanisms concerned work, therefore, in a very prompt and perfect way. It

might be imagined that the chief action is due to the different illumination of the various sides, the less illuminated being also that in which the chromatophores are less expanded. It is, however, easy to show that powerful illumination of the paler side does not alter the result. Nor is the lesser expansion due to any effect of contact or pressure on the chromatophores themselves. The effect of these factors would then be directly the opposite, namely, an increase in the expansion. The effect goes through the nervous system, it appears, not only on the chromatophores touching the ground or the wall, but on the entire half upon part of which the animal lies. It seems safe to conclude, therefore, that from the part of the body upon which the animal lies impulses are sent to the centers of the chromatophores of the corresponding area of the skin, the effect of which is a diminution of the "tonus" of the centers themselves. This fact adds a new and important link to the series of factors acting on the chromatophores and gives us a new instance of postural reflexes, and one belonging to a new class.

We are now in a position to form an opinion, as clear as present day knowledge allows, about the external factors influencing the chromatophores. A very simple experiment, however, namely, the section of any nerve going to the chromatophores, will show us that when these are separated from the central nervous system, none of the stimuli quoted above will act on them in the described way. We are therefore bound to conclude that all these stimuli influence the chromatophores through a nervous mechanism, which means that in reality they are not acting on the chromatophores themselves, but on their nerve centers. The time has come to say something about the centers themselves. It is not a part of our task this evening to expose at length the anatomical facts regarding the central nervous system of the cephalopods. It need only be remembered that it is the most highly organized and the most centralized among those of the invertebrates, the various ganglia running together to form a sort of brain. The importance of this is such that it has developed a protecting device,—a sort of cartilaginous skull. This "brain" is large enough to allow an experimental analysis of its various parts by means of the classical methods of stimulation and extirpation. In relation to the chromatophores, this analysis has gone so far as to show the existence of several centers for the chromatophores of the different parts of the skin. While these centers are located in different parts of the brain and are of a motor kind, Phisalix (1892, 1894) was able to demonstrate the existence in the highest ganglia, the so-called cerebral ganglia, of an inhibitory center acting on the chromatophores of the same side.

This interesting observation by Phisalix was not confirmed until a few years ago I undertook the task of examining the whole question of the central control of the chromatophores. In the study of this problem I availed myself not only of the classical methods of analysis by stimulation and extirpation, but also of the finer one offered by the use of drugs. I was then, and I am now, very well aware of the dangers connected with this method, and I readily acknowledge that its results are subject to criticism and are to be accepted with more reserve than those obtained by the former methods. At the same time, however, I am of the opinion that the results obtained by means of pharmacological analysis, especially if checked with those obtained by some other method, afford a very valuable help in carrying the analysis to a point where neither the scalpel nor the electrode, however delicate and however skillfully handled, could arrive.

As a result of this study, I am now able to confirm the data of Phisalix and to add something to them (Sereni, 1928*a*). The chromatophores are ruled by three different kinds of centers, all of them situated in the brain. The first kind are the purely motor (*B*) centers. They are located in the different subesophageal ganglia, are distinct for the two sides and for the different areas of the skin, and they cannot substitute for each other. These centers are overruled by a general coloration-center (*A*), probably to be located in the so-called central ganglia, and by an inhibitory center (*C*), corresponding to that of Phisalix, in the cerebral ganglia. Both these centers, the general coloration and the inhibitory one, although symmetrical, may act also on the opposite side, and the center on one side may permanently substitute for the other. Their relations to the purely motor (*B*) centers may be considered as similar to those between the cortical and the medullary centers in the vertebrates.

This description makes it perfectly clear, I hope, that the nervous mechanisms connected with the chromatophores are very complex indeed. It is interesting to observe that, in this case as in many more, the exactness and the delicacy, as well as the rapidity and the adequacy of the regulation, has been attained in the same way, namely, by the existence of a double innervation, excitatory and inhibitory. The ways and the methods nature employs in its laboratory are not so manifold as we sometimes fancy they are.

These have not been the only results achieved with the pharmacological analysis. When studying the action of the various drugs on the centers just described, I noticed (Sereni, 1928*b*) that they could easily be grouped, according to their action, into two classes. In the first were the substances producing an expansion of the chro-



matophores; in the second those inducing a retraction. This depends on the action of the different drugs upon the one rather than upon the other center. There is nothing astonishing in it, nor in the fact that the same effect, for instance, the expansion, can be attained either by a stimulation of the excitatory center or by a paralysis of the inhibitory. It is, however, rather unexpected to find that the grouping of the drugs corresponds very exactly to that with which we are well acquainted from the pharmacology of vertebrates, in sympathetico-mymetic and parasymphetico-mymetic drugs. The fact is that the former ones, for instance adrenalin, increase the expansion of the chromatophores, that is, the "tonus" of their centers; the latter ones, for example pilocarpin, decrease it by stimulation of the inhibitory center, which, on the other hand, is paralyzed by atropin.

The significance of this observation is increased by the fact that the similarity in the action of the drugs of each group among themselves does not limit itself to the action on the chromatophores, or, better, on their centers, but extends to many more functions. We may quote, for instance, the function of the ink-bladder, that of the iris, the respiratory action, and the activity of the muscles in general. On all these functions the drugs of each group act in the same way; the two groups, however, in opposite directions. Since the similarity with the action on the chromatophores is as exact as could be desired, we may conclude that all these functions are ruled by a double set of centers, excitatory and inhibitory, on each of which acts one of the two classes of drugs described above.

It can be added that the similarity in the grouping of the drugs according to their action in the vertebrates or in the cephalopods is a very exact one, not only in the broad lines, but also in particulars. All this seems to show that this is not due to chance.

There is, however, a difference, and, apparently, a very deep one, between the vertebrates and cephalopods. It is a well-known feature of the action of the vast majority of the drugs just named that in vertebrates they act at the peripheral end of the nerve; chiefly in some part of the junction between nerve and effector organ. In the cephalopods, on the other hand, all drugs act, at least in regard to the actions we are now considering, on the central nervous system. This seems to be a very deep difference, and a very important one too. On the other hand, I may say that this difference, however important it looks, could be expected beforehand. As a matter of fact, and as we shall see more clearly in a later stage of our examination, the conditions existing between nerve and effector organ in the cephalopods differ very widely from those in the vertebrates. For

example, no morphological differentiation such as the motor end-plates has been detected so far in the muscles of the cephalopods; nor does curari act on the latter in its usual and so characteristic manner. These observations may help to explain the reason for this one great difference among so many similarities. On the other hand, we must remember that the methods employed simply allow us to tell that the different drugs act on the central nervous system, or, better, on parts of it. With them, however, we are unable to determine the nature of the elements on which the drugs are acting, and it is quite possible that some of them at least do not act on the cells proper, but rather on the synapses, that is, on the nerve endings of the cells connected with those of the center on which the drugs seem to act.

Whatever may be the conclusion on this point, it cannot be denied that the pharmacological affinities in the vertebrates and in the cephalopods are very similar indeed, and this striking similarity cannot be without some deeper meaning. We know that in the vertebrates two at least of the substances acting on the two sections of the autonomic nervous system, namely, adrenalin and choline (or acetylcholine) are to be found in the body itself. These same substances are among those acting (with opposite results) in the cephalopods. The next step was therefore to look for the presence of these same or of some related substances in the cephalopods. Neither adrenalin nor choline has ever been recorded in the cephalopods. There are, however, at least three substances, tyramine and histamine on one hand, betaine on the other, whose action in the cephalopods is very similar to that of adrenalin and of choline respectively. These substances have been shown to exist in the body of the cephalopods, and the two first to circulate in the blood. We have, therefore, a very close correspondence with the facts in the vertebrates. In both cases substances exist in the body of the animal and in its blood which are pharmacologically active on the animal itself. It seemed rather improbable that this occurrence should be only casual, and it was worthwhile to try to find out if the circulating substances have any physiological importance. A first approach to the experimental solution of this problem was made possible by a fortunate occurrence. There are two very closely related genera, *Eledone* and *Octopus* and two species of *Octopus*, *macropus* and *vulgaris*. Of these *Eledone* and *Octopus macropus* are normally much more coloured, *Octopus vulgaris* much paler. In other words, in the first two normally the general coloration-center is dominant; in the third the inhibitory one prevails. If now the blood of *Eledone* or of *Octopus macropus* is injected in the

blood stream of *Octopus vulgaris*, the latter becomes for a while much more coloured, and on the other hand, if the blood of *Octopus vulgaris* is injected in *Eledone* or in *Octopus macropus*, they become paler (Sereni, 1929a). The same experiment may be performed by connecting the two animals in crossed-circulation preparation which I have devised for this purpose and which has been described elsewhere.

The results of this simple experiment show in a very clear and definite manner that the circulating blood has some property of modifying the conditions of the centers of the chromatophores. That this property in the case of *Eledone* and *Octopus macropus* is connected with the presence of tyramine or histamine, or both, is made probable by the fact that these substances are much more abundant in the blood of *Octopus macropus* and *Eledone* than in that of *Octopus vulgaris*. The probability becomes a higher one by the demonstration that in conditions when, after an experiment of Bottazzi's (1924), tyramine or histamine are known to be secreted in the blood, the expansion of the chromatophores increases. The final proof was given recently (Sereni, 1929b), when, by the extirpation of the so-called posterior salivary glands, (which, as the result of the work of Henze and Bottazzi, are known as the organs where tyramine and histamine are chiefly produced or accumulated) I was able to induce in *Octopus macropus* and *Eledone moschata* a condition of complete paleness and general atonia. This condition persists and the animal eventually dies, but it is possible to correct this state by the injection of tyramine and histamine or of the blood of a normal animal.

The work just described proves beyond any reasonable doubt that what we had guessed as probable, namely, that the presence in the body of pharmacologically active substances could not be without some physiological significance, is true. It gives at the same time the first definite and, I daresay, conclusive proof of the existence of phenomena of internal secretion in the cephalopods and in the molluscs in general and one of the very first demonstrations of the same phenomena in invertebrates.

Let us now, after this rather long, but necessary, parenthesis, go back to the physiology of chromatophores. From this standpoint the results quoted above give us the final link in the long chain of phenomena connected with the regulation of the chromatophores. The centers in the central nervous system, controlling the chromatophores (as well as those connected with many other functions) do not only respond to external factors like those previously described; they are also under the continuous influence of internal, humoral factors. These produce and maintain the tonic basal condition, on which the

relatively quick changes induced by external factors take place. The effect of these different controls is to make the regulation much more delicate.

There is another point I should like to bring to your notice. It has been the merit of Redfield to show, some years ago, that the chromatophores of a lizard are subject to a humoral control. His results have been widely confirmed and extended in regard to the chromatophores of the vertebrates (Hogben, 1924). Now my results proved that similar phenomena occur also in the control of the chromatophores of the cephalopods, and almost at the same time Koller (1928) and Perkins (1928) were able to show that the same holds for the chromatophores of the crustaceans. There is one difference, however, between the working of the humoral control in the vertebrates and in the cephalopods. In the vertebrates it acts on the chromatophores themselves; in the cephalopods, on the other hand, the humoral control works through the nervous system. That means, it influences the centers controlling the chromatophores. The explanation of this difference may most probably be found in the much higher complexity and development of the chromatophores in the cephalopods.

I feel that this lecture has already been long enough, and I will not detain you much further. Our survey of the physiology of the chromatophores would be, however, very incomplete indeed if, after considering them from the standpoint of their regulation, we should not examine them for themselves. In the first place, when in connection with the central nervous system, the chromatophores are more than anything else to be considered as a sort of indicator, and a very valuable and suitable one, of phenomena happening elsewhere, chiefly in the central nervous system itself. It is possible, however, to consider the chromatophores—or better, in this instance, their radial fibers—from the standpoint of muscular physiology. The chief interest of this study lies in the fact that we have to do here with single muscle-fibers such as there are not many opportunities to find elsewhere.

All the experiments which I have described heretofore have been performed on chromatophores connected with the central nervous system through nerves. From now on we shall consider experiments on the chromatophores deprived of this connection. It would be, however, a very misleading procedure to consider the chromatophores of every part of the skin whose nervous connections have been previously severed as deprived of every nervous influence and therefore showing simply the properties of the muscular fibers alone. The skin is so rich in nervous branches, connected to form different plexi, that

whenever a stimulus is applied, it is through the nerves that the excitation reaches the muscles. This is the conclusion derived from the character of the responses, which are identical with those produced by the stimulation of the nervous trunks. When, however, enough time is allowed to pass after the section of the nerve, or when the animal has been dead for some time (the actual length of time varying in both instances with the season, that is to say, with the temperature), the phenomena observed after stimulation and also without stimulation become very different. First of all, while in the first days after the section of the nerve, or in the first hours after death, the skin is completely pale, as well as motionless. Later on it begins to show some coloration, and we notice on it the wonderful phenomenon of the so-called cloud-migration (*Wolken- or Wellen-wandern*), which may be described as an almost continuous and uninterrupted wandering from one spot to the other of a condition of expansion of the chromatophores, that is to say, of contraction of their radial fibers. It is characteristic of this phenomenon that the contraction, during its wandering, never jumps; it always goes from one chromatophore to the next, and never to one further away. This fact is of the utmost importance for the explanation of this phenomenon, which is completely different in its nature from anything we may observe on the normally innervated skin. In the latter case you may now and then observe something superficially resembling the phenomenon described above, but in this case the expansion appears here and there in completely separated territories, which, if the observation is accurate enough, may be recognized as those innervated by the same nerve, or group of nerves. In this case the contraction of the radial fibers is the result of an excitation of one or more nerves; in the first one we have to do with a purely muscular phenomenon, as we shall very soon see.

As a matter of fact, if a stimulation is sent to the skin on which the "cloud-migration" is observed, either through the nerves or directly, the phenomena produced are very different from those normally observed. No effect whatever follows the stimulation of the nerves. When the electric stimulus is applied directly to the skin, we notice that the excitability of the chromatophores, to galvanic as well as to faradic currents, is very low. It is only with very strong currents and with very prolonged stimulations, that it is possible to get an effect. Even in this case the expansion is limited just to the few chromatophores lying between the electrodes. It starts only after a prolonged delay (often after the stimulation is over) and lasts longer than the stimulus. This effect is not comparable to the one observed

on the normal skin, where the direct application of any stimulus produces an immediate and diffuse expansion of the chromatophores which lasts just as long as the stimulation itself. The electric excitability in the two conditions is very different. On the other hand, in the skin where this modification of the electric excitability occurs, the mechanical excitability of the radial fibers of the chromatophores is very high, much higher than when the innervation is intact. This augmentation is accompanied by an increase of the excitability for luminous and perhaps also for chemical stimuli. This increase of the mechanical excitability makes possible the explanation of the phenomenon of the cloud-migration. If the skin is left unexcited and in the dark, its chromatophores are at rest and it is pale, just like the normal skin. As soon, however, as a stimulus reaches any spot on the skin, the chromatophores of this spot expand; their radial fibers, it follows, contract. By their contraction, however, the fibers pull on the next ones; and as these are in a condition of mechanical hyperexcitability, this very slight stretching represents a stimulation strong enough to produce the contraction of the stretched fibers. In this way the contraction proceeds, giving the impression of a "wandering cloud." The same explanation makes it clear why, when any spot in the denervated skin is stimulated, the contraction very often propagates in concentric circles, or at least in the two opposite directions. On the other hand, it might seem strange that the contraction advances, that is, that it only goes one way and not back. It must be considered, however, that the radial fibers, like every excitable tissue, have a refractory period. The pull exerted by the fibers of the chromatophore which contracts, first reaches all surrounding fibers which are at rest, and therefore provokes their contraction. This contraction, like that of the fibers of the previous chromatophore, also exerts a pull, and therefore a stimulation, all round; but while on one side this stimulation reaches fibers which are at rest, on the other it reaches the fibers of the first chromatophore, which have just undergone their contraction and are therefore in the refractory period following the excitation. The stimulation given by the pull is therefore ineffective on these fibers and the expansion of the chromatophores extends always only to one side and not to the other.

The chromatophores of the skin which has been for some time deprived of its nervous connection are, as we have seen, the bearers of very remarkable properties, which are, at least apparently, quite different from those of the normally innervated chromatophores. The obvious explanation of this fact is that, after the section of the nerve, the latter degenerates, and the radial fibers, deprived of this control,

develop some new properties of their own. There are two more facts worth noticing in this connection. The first is the rapidity of the degeneration of the nerves after the section. Even in the coldest season, it never takes more than a week or ten days for the nerves to degenerate completely in *Octopus* and *Eledone*. The physiological results, which are in themselves very clear and conclusive (Sereni, 1929c), have been, at my suggestion, controlled by Young (1929) with histological examination. In the last few days I have been able to perform the same experiments here on *Loligo*, and I have been able to see that in this much more delicate animal the physiological aspects of the degeneration of the nerves are complete (in summer, at least) in not much more than twenty-four hours. After this time it also becomes impossible to stain the nerves going to the chromatophores with methylene blue. In this instance, as in those previously quoted, the physiological results are perfectly checked by the morphological ones. When one thinks of the length of time required for nerve-degeneration in the frog, there is hardly need to emphasize the difference between the two. This shows, I think, that the rapidity of the degeneration of the nerve after its section cannot be simply dependent upon the body temperature. On the other hand, when the nerve degeneration is complete, no more changes occur in the muscles, even after six to seven months.

The second point I want to emphasize is that almost exactly the same changes occur after the death of the animal as after the section of the nerves. The most outstanding difference (which helps to explain other ones) is that, in the former case, the changes occur much more rapidly—in the hot season in a few hours. It is very difficult to admit that, during this short time, great changes may occur in the muscle-fibers themselves, of the kind observed after the denervation of muscles in vertebrates. Since, however, the phenomena observed in the chromatophores and in the muscles of the cephalopods are exactly the same both after denervation and death, it is only left us to conclude that in neither case does "degeneration" of the muscle-fiber occur. The phenomena observed are much more those inherent in the muscle-fibers as such, unaffected by the nerves.

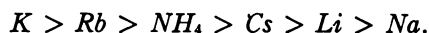
It is therefore on the skin of an animal whose nerves have been cut some days before, or which has been dead for some hours, that we must perform our experiments when we want to consider the chromatophores from the standpoint of the physiology of muscle-fibers. The radial fibers are smooth fibers, but in the rapidity of their contraction and relaxation they approach striated fibers. On the other hand, in their notable capacity for rhythmical contraction they re-

semble the fibers of the myocardium. Their study is therefore of much interest, the more so because when you experiment on the chromatophores, you are in reality doing many experiments at once. As a matter of fact, since every fiber contracts independently, the effect noticed is not, as in every other muscle, a summation of the individual effects of each fiber, but more like a statistical average of them. The result is, therefore, more likely to approximate the truth.

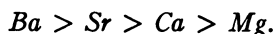
It is easy to observe in the chromatophores many phenomena regarding the general physiology of muscles. Cadaveric rigidity, for instance, reveals itself with an extreme expansion, which lasts for some hours and passes off slowly. It is preceded, during some hours, by a beautiful and very irregular play of colour.<sup>2</sup>

In addition, it is possible to study the action of many factors on the chromatophores, with the advantage, already emphasized, that you can be sure that the effect observed is due to an action on the muscle-fibers themselves. I have tried the effect of high and low temperature and of changes in the osmotic pressure (Sereni, 1927). There are beautiful researches by Hertel (1907) on the action of lights of different wave-length which affect differently the various kinds of chromatophores. By the way, since the colour belongs to the chromatophores proper and not to the radial fibers, this fact shows that between the former and the latter, relations exist which cannot be nervous, since the experiments of Hertel were performed on paralyzed skin.

Among the advantages of the chromatophores in comparison with other objects for the study of muscular physiology, one of the more important is that, since the fibers are isolated and very superficial, they are all reached and affected practically at the same time. Consequently there is no lagging behind. This advantage is especially noteworthy in the experimental analysis of the action of chemical substances. I have tried the action of different cations and anions (Sereni, 1927). All of these, in solutions isotonic with sea water, induce a more or less intense and rapid contraction of the radial fibers. Among the monovalent cations the most active is *K*, the least *Na*; between these two extremes other cations act as follows:



*Ba* is the most active of bivalent cations; the others are ordered as follows:



While *Na* produces a very distinct contraction, *Mg* has only a

<sup>2</sup> This phenomenon was demonstrated by means of cinematograph film.



short and indistinct action in this sense, and its chief effect is to produce a complete but reversible paralysis. It is *Mg* which antagonizes both the *K* and *Ca* in sea water in regard to their action on the chromatophores, and no solution without *Mg* is able to maintain the latter in good working condition for any length of time.

Among the anions the series, which is less distinct, is as follows:



It would take us too far to compare these series with the similar ones obtained on other objects and especially on smooth and striated muscles, and we must be satisfied with this simple hint.

The majority of these ions produce exactly the same effect on the normal and denervated skin. This means that they act on the muscle-fiber proper. Since they act also when the fibers have lost their direct excitability, they probably act on the contractile mechanisms themselves. While the ions act peripherally, none of the drugs I have tried, with the probable exception of histamine, act at all under these conditions; we can therefore conclude that they do not affect the contractile mechanisms, on which the ions act. If we experiment with the same drugs on skin which has been denervated for a very long time, only a few of them, namely strophanthin and digitalin, show their action, which consists in a powerful and very durable contracture. Since, under these conditions, the radial as well as the other muscular fibers have lost their indirect, but still retain their direct excitability, the conclusion is that strophanthin and digitalin act on the excitatory apparatus of the muscle-fiber.

On the contrary, on the normal, freshly isolated skin it is possible to evoke an intense expansion of the chromatophores (that is to say, a contracture of their radial fibers) not only by different ions or by strophanthin, but also by many other drugs, such as nicotin, acetylcholine, choline, pilocarpine, arecoline, coniine, etc. In the normal skin many nerves and even nervous plexi (without ganglion cells) are to be found; and it seems at first very likely that, since the last-named drugs do not act directly on the muscle-fibers, they evoke the contraction of the latter through a stimulation of the nerves. It is possible, with a little skill, to isolate a fairly long piece of some nerves, for instance, of the coloration nerves of the tentacles or of the mantle-connectives. If now any of the above-named drugs, say nicotine, is allowed to act on this isolated nerve, but not on the chromatophores, no contracture occurs. The nerve may or may not lose its excitability after more or less time, but there is no sign of any stimulation. It seems highly improbable that the action of drugs on different parts

of the nerve-fibers is different. It appears, therefore, safe to conclude that nicotine and its fellow-drugs do not produce the contracture by acting on the nervous plexi of the skin. There is another fact which makes this conclusion quite certain. We have seen above that the degeneration of nerves begins after a very short time and is complete a few days after the section. Now, after this delay, while the indirect excitability of the chromatophores is completely and forever lost, nicotine still maintains the capacity for producing an intense contracture. The seat of its action cannot, therefore, be in any part of the nerve, which at this time is completely out of function, but must be more peripheral. On the other hand, we have seen that nicotine and other similarly acting drugs do not act on the muscle-fibers proper.

There is only one solution to this puzzle, namely, that these drugs act neither on the nerve nor on the muscle-fiber, but on something intermediate, on something which does not belong to the nerve, because it does not degenerate with it, but is strictly dependent on it for its function. This dependence is shown by the fact that when the nerve has degenerated, the excitability of the muscle-fiber for electrical and mechanical stimulations is the same in the first months, when the intermediate structure is still there, as later, when it also disappears. The excitability for nicotine and other specific chemical stimulants, which is still present in the first period after the nerve-degeneration, disappears after some months. It is just this difference which allows the conclusion that between the two periods there must be a change, namely, the presence or absence of the intermediate zone on which nicotine acts (Sereni, 1930).

There is hardly need to recall how far these facts correspond to the well-known phenomena which occur after nerve-section in the vertebrates. They are the more interesting because they represent the first experimental examination of these problems outside the vertebrates. As in cephalopods, also in vertebrates, nicotine acts on a part intermediate between nerve and muscle, a part which does not degenerate with the nerve but disappears only much later, very likely, as in cephalopods, because of a sort of *atrophia ex non usu*.

There is, however, a difficulty in connecting and comparing the phenomena in vertebrates and in cephalopods. In the first group the study of these phenomena is very much helped by the existence, in curari, of a drug which antagonizes the action of nicotine and paralyzes the motor nerve-endings. Now, as mentioned above, curari does not show its specific action in the cephalopods at all, nor does it in any way interfere with the action of nicotine. It was just because of this failing of curari that up to the present nothing was known about the connections between nerve and muscle in cephalopods.

It must be added that so far the most painstaking examination, in the hands of such careful workers as Hofmann (whose results in this regard I can fully substantiate), has not succeeded in showing any differentiation of the motor nerve-endings in the cephalopods of the type of motor end-plates. It is on these latter, however, that curari most probably acts in the vertebrates, and their absence is in good accord with the absence of action of curari.

On the other hand, it must not be believed that, because curari fails to show its characteristic action, it is impossible to find in cephalopods any drug which specifically paralyzes the same zone which is stimulated by nicotine. By a systematic experimental investigation of the action of many drugs on the muscles of cephalopods, I was able (*loc. cit.*) to find that strychnine more characteristically and specifically, but also quinine, caffeine, ephedrine, tetrahydrobetanaphthylamine, act on the intermediate zone, which they paralyze, antagonizing in this way the action of nicotine and of the similarly acting drugs.

Things seem to become rather complicated, I fear; and complicated, indeed, they are. A possible key is given by the most recent work of Riesser (1925) on the vertebrates. This author has shown that, contrary to the opinion of Langley, it is quite possible to distinguish the action of curari as an antagonist to nicotine from the block of the conduction of impulses from nerve to muscle. The latter, which appears for minor doses, disappears after the degeneration of the nerve, and must be located in the nerve-ending proper. The former effect, which can be demonstrated a long time after the section of the nerve, although in the end it also disappears, has as its seat a more peripheral part, the receptive substance of Langley.

If we compare these results with our own in the cephalopods, we must conclude that the character of the differentiation we succeeded in demonstrating in the radial fibers as well as in all other muscle-fibers of cephalopods is similar, although not identical, to that of the receptive substance in the muscles of vertebrates. It cannot be said whether the physiological and pharmacological differentiation—the only one demonstrated by our results—corresponds to any morphological differentiation. That this last has not yet been found cannot be accepted as a definite negative proof, since, according to the opinion of Boeke, the morphological structure corresponding to the receptive substance should be his periterminal network, and it is quite possible that it has not yet been demonstrated because of technical difficulties. On the other hand, there are neither physiological nor pharmacological proofs of a differentiation in the cephalopods of the motor nerve-endings, corresponding to that on which curari acts.

This is in accord with the results of the histological research, which fails to demonstrate any form of end-plates (which could not be overlooked).

We have described the changes in the excitability of the chromatophores which follow the section of their nerves. We have not, however, or only too briefly, referred to the fact that, soon after this section, the chromatophores expand spontaneously, developing what has been called a "peripheral autotonus."

It is too late now to describe at length this phenomenon and to discuss its origin. I should only like to recall that betaine, which, as stated above, is to be found in the cephalopods, acts on their chromatophores exactly like nicotine. On the other hand, tyramine, whose presence and action on the nerve-centers has been described before, very much increases the excitability of the radial fibers of the chromatophores. The action of these two substances suffices, therefore, to explain the phenomena (expansion, increase of the mechanical excitability) observed on the chromatophores after the section of their nerves. As they are to be found in the body and the blood of the cephalopods, it seems not too difficult to admit that they play a part in the production of these phenomena. Therefore, while on one hand they control the behaviour of the chromatophores through their nerve-centers, on the other they act on the chromatophores themselves, but this peripheral influence can only be shown when the central control has ceased to act for some time.

*Ladies and gentlemen:* At the beginning of this lecture, I told you that the study of the chromatophores of the cephalopods has many interesting aspects, and that they can be examined both for themselves, when their radial fibers supply us with an almost unique opportunity of studying under the best experimental conditions the reactions of physiologically isolated muscle-fibers, and as indicators of phenomena taking place in their ruling centers.

I hope that the results presented may have substantiated these claims. To summarize them in a few lines, we can say:

1. The chromatophores, under normal conditions, are ruled by different groups of nervous centers, both excitatory and inhibitory.
2. These centers are under the continuous control of at least two humoral factors, whose action determines the "tonus" of the nerve-centers, and by this means, the condition of the chromatophores themselves. This is the first demonstration of humoral correlations in cephalopods, and it is very likely that it is connected with an endocrine function of the posterior salivary glands.

3. The connection between nerve and muscle has for the first time been studied in cephalopods, and I was fortunate enough to show that there is a differentiation, whose characteristics are similar to those of the receptive substance in the vertebrates. There seems to be no differentiation corresponding to that on which curari acts.

These are only a few instances of the many experiments for which the chromatophores of the cephalopods offer an admirable object. I wonder if somebody may perhaps ask what is the purpose of repeating on different objects experiments already performed. It may be that this is sometimes an idle and useless task; although, you must remember, it has happened more than once that repetition of former experiments on a new object has led to quite new results, not infrequently more important than the first ones. But I daresay there is one more reason, and a very good one, for welcoming every addition to the number of our experimental animals.

Physiology today is moving in the direction of general physiology. Now we are perhaps, all of us, too often inclined to forget that a general physiology cannot be only a general physiology of vertebrates, but has as its necessary base and complement comparative physiology. On the other hand, to compare the physiology of different animals, we must study the physiology of each of them. To say it in different words, general and comparative physiology can only rest on an ever-widening knowledge of the special physiology of the different types. It often happens that this widening is accompanied by an increase and a deepening as well; if not, the results will be welcome just the same.

It may appear that I am rather too keen on this point. I hope, however, that this will not be your opinion, ladies and gentlemen, who have gathered from all over the world at this place, which is, undoubtedly, together with its sister Station in Naples, the chief shrine where comparative physiology and general physiology have been honored and practiced daily for many years and, *favente Deo*, will be for many years to come.

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