GALVANOTROPISM AND "REVERSAL OF INHIBITION" BY STRYCHNINE.

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I.

Definite neuromuscular effects are produced in a variety of organisms by the passage of a constant electric current. The character of the induced galvanotropic curvatures and movements has played a certain part in the development of the tropism doctrine (*cf.* Loeb, 1918), but these reactions still await quantitation—which is difficult—as well as fuller utilization for the analysis of animal movements. The interpretation of galvanotropism in metazoans turns at present upon the necessary and sufficient assumption that the current serves to excite definite groups of nerve cell bodies (presumably as determined by the axial orientation of these cells with respect to the polarity of the current—Loeb and Maxwell, 1896; Loeb, 1918; Moore, 1922–23, *b*).¹ The effects are such that among annelids, for example, the animal typically extends and lengthens when the head is toward the cathode, but shortens when the current is in reverse direction. These effects are in certain respects similar to those produced by neurophil drugs. Strychnine, for example, induces a similar and comparable elongation, whereas nicotine leads to shortening. It was considered that if the effects of such alkaloids are indeed due to selective or differential unions with particular groups of nervous elements, the result of combining the action of a substance of this type with that of the electric current should be merely an accentuation of the primary action, since the response induced involves ac-

¹ It should be noted, however, that the apparent impermeability of cells to direct current imposes certain conditions on the form which this interpretation may take.

tivity of the same nerve-muscle groups. In certain cases this turns out to be true.

The effect of strychnine is of especial interest. In the spinal cord of vertebrates it is usually supposed (Starling, 1926) that the strychnine effect is due to abolition of the inhibitory component of normal coordination, so that the inhibitory effect is transposed into an excitatory one. Coordination thus becomes impossible, for antagonistic muscles contract together, so that the resulting movement is determined by that muscle group which is the stronger. The locus of this action is commonly assumed to be at synapses (or their homologues), but until the nature of inhibition is better understood it might be assumed that the strychnine effect may really involve the elective excitation of nerve cell bodies.

Reversal of reaction by strychnine is apparent in a variety of invertebrates² in which the coordination of antagonistic muscle groups is requisite for normal movements. If the effect of the strychnine is primarily one depending upon the elective excitation of particular nerve cells, then a strychninized annelid, in which the longitudinal muscles are relaxed, circular muscles contracted, so that the animal is much elongated, should be caused to elongate still further when submitted to the action of a constant current with cathode at the head. The fact is that under these conditions the usual cathodic galvanotropism is reversed; the animal shortens when the head is toward the cathode, elongates when the head is toward the anode. The simplest interpretation of this result is that the reversal effect of strychnine is a true synaptic effect not explained by elective excitation of nerve cells, and the experiment therefore becomes a test of the nature of at least certain types of reversal of inhibition.

It is proposed to illustrate this point by means of tests with platyhelminths, nemerteans, and annelids.

II.

Observations on the galvanotropism of common shore nemerteans were made in glass troughs containing sea water, through which a current of about 4 volts, 0.5 milliamperes per sq. cm., was sent

²*Cf.* Moore, 1918-19, 1919-20; 1923-24, *a.* Crozier, 1919-20, 1922.

by means of cotton pads. The current density was varied by changing the cross-section.

Several species of *Lineus* are pronouncedly cathodic. Most of the experiments were made with *Lineus socialis*; similar results were also obtained with *L. viridis*, a species of *Tetrastemma*, and another of *Cerebrotulus*.

In creeping the body is moderately elongated, and there is frequently no evidence of peristaltic waves; sometimes a succession of such waves may be apparent. When a current is sent through the water the nemertean undergoes marked elongation if the head is toward the cathode, and creeping continues in that direction. If the current is reversed, the body promptly shortens, and the anterior end, now toward the anode, becomes notably shortened and thickened; the worm may then move (backward) toward the cathode, but soon the anterior end extends to one side and both ends of the body are then directed to the cathode. Continued creeping brings the nemertean into cathodic alignment with the current. If at first the animal is transverse to the lines of current flow, both ends are at once directed to the cathode, the body becoming bent in a U. This behavior is essentially like that of the earthworm (Moore and Kellogg, 1916; Hyman and Bellamy, 1922; Moore, 1922-23, *b*) and is shown by other annelids, *Nereis* (Hyman and Bellamy, 1922) and *Harmothoë*, as well as by marine and fresh water planarians. If the current be sent through the nemertean dorsoventrally, again the longitudinal muscles on the cathode side contract and produce the U-posture. As in the case of the earthworm (Moore, 1922-23, *b*), pieces or regions of the body exhibit similar responses. If a portion only of the intact nemertean be suspended in the current-trough certain differences appear. With the anterior end submerged and hanging vertically, closure of the current results in cathodic orientation and elongation of the submerged part; reversal of the current produces extensive shortening, so that the immersed part is lifted out of the water. Immersion of the posterior end only shows that part orienting with the posterior tip toward the anode, and reversal leads to contraction of the longitudinal muscles.

Isolated anterior ends of *Lineus* orient cathodically and elongate in this direction, shortening and turning on reversal. A transverse

current produces the U-form. The behavior of isolated posterior parts is somewhat more complicated. Longitudinal muscles contract on the cathode side,—except occasionally just after cutting, when those on the anode side contract,—so that the typical U effect of a transverse current is produced; and the contraction of circular and dorsoventral muscles, with relaxation of longitudinals, leads to the usual elongation when the posterior end is toward the anode, which is changed to a shortening and thickening of the piece when the current is reversed. But peristaltic waves are commonly initiated at whichever end of the body happens to be directed toward the anode; and the piece creeps toward the anode whether the creeping be posteriorly or anteriorly directed; in a number of preparations it was clear that when in a U, with the two ends toward the cathode, peristaltic waves began at the anodic bend and moved, more or less alternately, to either end. Elongation of the fragment, however, occurs only when the posterior end is toward the anode, and reversal of the current produces the usual shortening and thickening; creeping with the anterior toward the anode is very slow, with faint, well spaced peristaltic waves and some wrinkling due to local circular muscle contraction. Occasionally, if antero-posterior peristalsis has been initiated it persists on reversal of the current, so that the piece creeps to the cathode. The behavior of pieces cut from the mid-region of the body, and thus with two cut surfaces, is similar. These, and the other isolated parts, were usually kept for about 24 hours before being tested.

The long proboscis of *Lineus* is not easily discharged.³ Since the proboscis contains circular and longitudinal muscles it was desired to test its galvanotropism apart from the control of the nervous system in the body of the worm. The extracted proboscis shortens when its distal end is toward the cathode. Short fragments show longitudinal contraction on the cathode side. In thus paralleling the behavior of the whole body the proboscis is similar to the isolated tentacles of *Polyorchis* (Bancroft, 1904). It is an interesting question, however, as to the possibility of independent galvanotropic

³ The proboscis of *Tetrastemma* was occasionally found to be everted when the anterior end was turned toward the anode, and retracted when this end completed its cathodic orientation.

behavior of the parts of an animal. An illustration is afforded by the ceratia of eolids. *Aeolida diversa* is cathodic, as are other nudibranchs which have been tested. *Aeolida*, and *Dendronotus arboreacens*, elongate when the anterior end is toward the cathode; the tentacles and "rhinophores" bend to the cathode, but the ceratia (all but the most anterior four or six, in *Aeolida*) bend to the anode. Reversal of the current causes retraction of oral and dorsal tentacles, shortening of the body, and the ceratia on the posterior part of the body stand out sharply and turn toward the anode. If the current is transverse, the animal shows extension of the ceratia only on the anode side. When autotomized posterior ceratia are studied it is found that they shorten when the distal tip is toward the cathode, elongate when the base is at the cathode; a transverse current produces longitudinal contraction on the anode side, so that both ends point to the anode. This effect cannot be due merely to the absence of the central ganglia because both anterior and posterior halves of transversely bisected individuals are found to creep and to orient cathodically. A further illustration of this sort of difference between the behavior of the body as a whole and of an appendage is found in *Echiurus*.

The galvanotropism of *Echiurus chrysacanthophorus* was of especial interest for the experiments in view, because the orientation is anodic. A current sent transversely through the body leads to longitudinal contraction on the anode side, the body being thrown into a U. As the oral end turns to the anode, the circular muscles contract and this end elongates. Both normal and small regenerating proboscides, however, extend toward the cathode. With the body lengthwise in the current elongation is shown, particularly at the oral end, when the oral end is toward the anode; the proboscis is turned back toward the cathode. Peristaltic waves run from oral to aboral pole. When the current is reversed, the aboral end, now anodic, elongates markedly, and the oral end swings around so as to be directed to the anode. In some cases, locomotion being difficult on the smooth glass bottom of the trough, the animal progresses toward the cathode. Attention was chiefly given, however, to the interplay of circular and longitudinal muscles as the animal lay lengthwise to the current. Here it is obvious that longitudinal contraction is determined when the oral end is toward the cathode, longi-

tudinal elongation and circular contraction when the oral end points to the anode. The isolated proboscis, however, shortens and thickens, its lateral edges and the distal end curling together, when the cathode is at the base of the organ; current reversal produces uncurling and longitudinal extension. Thus the proboscis may be considered intrinsically cathodic in its orientation, in contrast to the behavior of the body of the geophyean.

The foregoing refers to the orientation of *Echiurus* lying on the bottom of a glass dish. At night, however, as described by Wilson (1899-1900), the worm can swim in an aquarium by means of vigorous peristaltic movements of the body wall; this performance is very striking in view of the mud-dwelling habit of the worm. When actively swimming *Echiurus* orients and swims to the cathode. Swimming movements were excited by repeated rapid reversals of the galvanic current, and orientation was then cathodic. Reversal of normally cathodic galvanotropism has been noticed in *Nereis* long confined to the laboratory (Hyman and Bellamy, 1922), but this is not the explanation of the behavior of *Echiurus*.

III.

The usual effect of strychnine upon flatworms and annelids is to produce (Knowlton and Moore, 1917; Moore, 1918-19) an enhanced excitability, and a reversal of the usual reaction to a local touch, for example, so that the contraction of longitudinal muscles is replaced by extension and the contraction of their antagonists. It is commonly observed that the gross effect of nicotine is to produce a shortening, thickening, or ventral flexure of the body of worms and other invertebrates (*cf.* Moore, 1919-20; Crozier, 1919-20; Crozier and Federighi, 1924-25). In the flatworms, nemerteans, and geophyean used in the present experiments no unusual effects of strychnine or of nicotine were obtained, but it is necessary to record the results briefly, for the interpretation of the tests with the galvanic current.

Lineus in aquaria tends to collect in dark corners, but its negative phototropism is not pronounced except when first collected. The initial noticeable effect of treatment with strychnine sulfate (1:50,000) is the marked enhancement of this phototropism. Later a more or less stationary transverse enlargement of the body is apparent, at the

level of the mouth; the lips are widely separated and the pharynx open. This is succeeded by the addition of more "standing waves", posterior to the mouth level; soon the whole body becomes much elongated. With higher concentrations the anterior half of the animal ultimately swells due to relaxation of the circular muscles as well as of the longitudinals, and the whole body is quite flabby. In solutions as concentrated as 1:2,000 the posterior region is very much attenuated, and the constriction of circular muscles pinches the body into fragments. Before this happens the body may be thrown into a coil, and in this the *dorsal* surface is always contracted, so that the animal may be said to be opisthotonic.

In the stage of excessive elongation, which with proper concentration of the drug lasts for some hours, creeping is entirely normal, but the reactions to tactile stimulation are reversed. A touch with a needle on the dorsal mid-surface of a normal *Lineus* leads to quick circular constriction at that level, coupled with longitudinal muscle contraction immediately anterior and then posterior; the two contractions of the longitudinal musculature run as waves in either direction; the circular constriction at the point touched persists for a time which varies with the severity of the excitation. If the animal be lifted from the bottom with a needle, it shortens, thickens, flattens; then the anterior end is extended and the circular constriction there begun progresses posteriorly.

A strychninized *Lineus* exhibits a stage in which the longitudinal shortening occurs anterior to the level of a touch, but the posterior part shows only a prompt lengthening. Then a little later it is seen that a gentle touch produces, not local circular constriction with attendant longitudinal contractions, but a local shortening *after* a local extension. The full development of this phase of the strychnine effect finds the animal responding to a touch by a violent contraction of the circular muscles, so that the whole body is enormously thinned and elongated. This reversal is also shown when the anterior end, with its ganglia, has been amputated, and indeed by short posterior pieces. At no time, however, does the animal spontaneously creep backward.

The two points which are of interest in the present connection are (1) the general elongation of the animal by strychnine, comparable

to the galvanic effect with cathode at the anterior end, and (2) the reversal in the action of circular and longitudinal muscle groups following local stimulation.

There is one pertinent mode of response which does not seem to be affected by strychnine, at least until creeping is no longer possible. According to Moore (1923-24, *b*) the homostrophic reflex which tends to maintain the pursuit of a straight path in annelids (Morgulis, 1910; Moore, 1922-23) and in some arthropods (Crozier and Moore, 1922-23; Crozier, 1923-24) is not exhibited by *Cerebratulus*. The interest of this fact comes in connection with the suggestion (Moore, 1923-24, *b*) that the reflex excited by a lateral curvature of the body and which results in the anterior part being brought into alignment with the tail, might in some way be connected with segmental organization, since it is not seen in *Cerebratulus*, in planarians, or in the slug *Limax maximus*. But in a variety of the smaller nemerteans it is easily shown that precisely this homostrophic orientation of the anterior end is an important feature in directed creeping. Within a region extending back some 2 cm. from the anterior end typical homostrophic response is obtained in forward creeping, if the anterior end has been bent sharply to one side; slighter lateral displacements are effective nearer the anterior end. In backward creeping the posterior end continues to travel in the direction in which it is put, but the homostrophic adjustment of the anterior end is evident when this part of the animal travels backward through an impressed bend; the homostrophic orientation is therefore not dependent upon the direction of progression, but upon a central nervous state connected with the creeping act. "Beheaded" worms fail to show this behavior. The homostrophy of normal animals is accentuated by treatment with nicotine, but continues absent at other levels of the body.

IV.

When *Lineus* in the stage of strychninization characterized by elongation of the body and by reversal of the usual reaction to touch are subjected to the action of the galvanic current it is found that they *shorten* when the head is turned toward the cathode, *elongate* further if the anterior end is toward the anode, and that if placed

transversely both ends of the body are turned to the anode. There is thus complete reversal of the usual galvanotropic behavior.

No reversal of galvanotropism could be secured by varying the current density. But at a slightly earlier stage in the strychnine action it is found that the longitudinal muscles on the cathode side of an animal transverse to the current will be contracted, as normally, but that when the anterior end is thus turned to the cathode the whole longitudinal musculature contracts and the worm shortens. To bring this about it is sufficient merely to bend the anterior end at the level of the mouth, so that the "head" is toward the cathode, the rest of the worm being parallel to the current lines, with posterior end to the cathode. Cases are found, also, in which the body is bent in a U with both ends toward the cathode, but with the longitudinal muscles contracted on the anterior half of the body.

These observations speak for the localization of the mechanism of strychnine reversal at the anterior end of the body. When the anterior part is amputated, at a level slightly posterior to the mouth, the worm shortens when the posterior end is at the cathode, elongates when the current is reversed. This proves that the strychnine reversal in the intact *Lineus* is due to an effect upon structures at the anterior end of the body, and it is natural to conclude that the cerebral ganglion is the essential locus. The isolated "heads" elongate with anterior tip toward anode, shorten on reversal. The "head" region so defined agrees with that evidenced by the homostrophic responses.

Isolated posterior halves are found to orient cathodically, although the end toward the cathode is broader than the posterior end, toward the anode. When the current is reversed the circular muscles at first contract, producing anodic elongation, but the anterior end turns toward the cathode.

v.

The galvanotropism of *Echiurus* supplies a complementary case. Treatment with strychnine (about 1:20,000, or less) causes the body to become more cylindrical, the circular muscles relatively more contracted; the proboscis is quite extended. Placed transversely to the current, both ends are bent toward the *cathode*, and the proboscis

extended in this direction. Reversal of the current causes first the proboscis, then the oral end, then, less vigorously, the aboral end, to be swung toward the new cathode. In line with the current flow, the body extends when the oral end is cathodic, shortens when the oral end is anodic; in the first case the proboscis is extended, in the second bent back toward the cathode.

The galvanotropic behavior of the body muscles is therefore reversed by strychnine. But the conduct of the proboscis is unchanged. The isolated proboscis presents very much the appearance of a non-strychninized one, save that the distal end is kept more curled together and that its reactions are slower. Its behavior toward the galvanic current is not altered by the strychnine. The sort of independence of the central nervous system exemplified by the proboscis of *Echiurus* (and by the ceratia of *Æolida*) it is permissible to correlate with the fact that these organs are readily lost by autotomy.

VI.

These experiments began with the thought that the contraction of circular muscles, relaxation of longitudinals, characteristic both of the strychnine effect and of cathodal galvanotropism in *Lineus*, involve the activity of the same neuromuscular elements. The result showed that the typical effect of strychnine is not enhanced by the corresponding effect of the current, but that on the contrary the usual galvanotropic orientation suffers reversal under strychnine. The obvious conclusion is that whether or no strychnine "excites" by stimulation of nerve cell bodies, it must also, or perhaps primarily, have some other action which produces a true "reversal of inhibition"; and it is difficult to avoid the assumption that the locus of this reversal is in synapses (or their homologues). The results of removing the "brain" of *Lineus* strengthen this assumption, as does also the absence of the reversal in the proboscis of *Echiurus*. The fact that both the cathodal galvanotropism of *Lineus* and the anodal galvanotropism of *Echiurus* may be reversed by strychnine disposes of certain interpretations which might conceivably be put upon the behavior of one of these forms by itself.

A further test of this conception of the place of action of strychnine

nine in effecting reversal of reaction is given by experiments with nicotine. Most of these experiments were made with several genera of marine platyhelminths. Exposure to dilute nicotine solution in time causes the longitudinal muscles of these worms to contract, the circular and transverse fibers to relax, so that the body is short and swollen; the ventral longitudinal muscles are so contracted as to arch the body dorsally. If the galvanic current, presumed to act upon nerve cell bodies, should produce normally a similar effect, a common locus for the action of current and of nicotine would have to be assumed. Nicotine does not reverse the cathodic galvanotropism of planarians, nor of *Lineus*. But, in nicotine solution, even before the characteristic picture of nicotization has begun to appear, the shortening, flattening, and arching of the body later produced by the alkaloid is clearly brought out during the flow of the galvanic current. The animals orient and move toward the cathode, but the body is broad and swollen, especially at the anode.

SUMMARY.

The cathodically galvanotropic orientation of nemerteans, *Lineus*, and the anodic orientation of the geophyean *Echiurus*, are reversed by the action of strychnine under conditions such that the typical "reversal of inhibition" induced by this substance is apparent. Nicotine does not give this result. Since it is necessary to assume that the strychnine effect is due to action upon the central ganglia, and since the galvanotropic effect depends upon action of the current on nerve cell bodies of the central ganglia, it must be assumed that the locus of reversal by strychnine is not perikaryal, but presumably synaptic.

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