THE NERVE NET OF THE ACTINOZOA

I. FACILITATION

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(With Seven Text-figures.)

INTRODUCTION.

THE most primitive form of nervous system in the Metazoa appears to be the nerve net, and in it undoubtedly many features of the nervous systems of the higher animals may be exhibited in a simplified form. But in comparison with such systems the nerve net seems to show certain far-reaching contrasts which suggest not merely a more primitive organisation but a fundamentally different method of transmission of excitation. It is our object to investigate the nature of the special properties of the nerve net. They may be summarised as follows:

(1) Excitation is conducted diffusely in all directions from the site of stimulus. This is well known from the classical experiments of Romanes (1885) on Medusae and of Parker (1918) on *Metridium*. There are, however, some evidences of polar conduction.

(2) The response varies in character according to the strength of the stimulus. Strong stimuli may call forth not only a more intense but an entirely different response from that of weak stimuli (Jordan, 1908).

(3) The greater the strength of the stimulus, the further is its effect propagated (von Uexküll, 1909, and others).

These properties seem to imply that excitation increases in intensity with the strength of the stimulus, and that this intensity falls off as the conducted disturbance travels away from the site of stimulation. That is, conduction takes place with a decrement.

So long as it was accepted that the nerve net consisted of an anastomosing network of fibres (Bethe, 1903) these properties were inconsistent with the nature of nervous conduction as revealed in the higher animals. More recently Bozler (1927) has shown, however, that at least in Medusae the nerve net consists of separate cells in simple contact with one another, without anastomosis. Conduction in the nerve net must therefore take place from one cell to another, and for this reason may not be comparable with simple transmission of excitation as found in the axon

of vertebrate nerve. But it remains to be shown that such cell to cell conduction will endow the nervous system with properties consistent with those just summarised. Bozler (1926) also found that the nerve net of Medusae possessed considerable differentiation. While certain tracts in the net possessed a marked decrement of conduction, there were others in which it might be argued that no decrement took place at all. The tracts possessing a decrement may show a marked tendency towards unidirectional or polar conduction, and Bozler stresses the analogy between the properties of these tracts and those of the synaptic nervous system. The fundamental question of the nature of the decrement, however, remains unsolved. In attempting to analyse this in the present experiments, one particular property of the nerve net has forced itself into evidence, that is the property of summation. Romanes showed that in *Aurelia* the property of summation of stimuli was developed to an unusually high degree. While the existence of summation has been frequently noted in connection with nerve networks, this property has never been systematically analysed; yet we shall see in this paper that it transcends all others in its importance.

The animal used in this work was chiefly the anemone *Calliactis parasitica* (Stevenson, 1928). It is the common large Actinian found commensal upon the shells of *Eupagurus bernhardus*. It is frequently referred to under at least two other names: *Adamsia rondeletii* and *Sagartia parasitica*.

MUSCULATURE OF *CALLIACTIS PARASITICA*.

To understand the responses of the Actinozoa it is necessary to remember that the mechanics of muscular contraction in these animals differs greatly from that in the Vertebrata. In the latter even the simplest movement of the limbs or body involves the complex reciprocal action of many muscles acting against a moving skeleton. On the other hand, the body of an Actinozoan is a muscular sac enclosing fluid which is chiefly responsible for its skeletal support. Movement of different parts of this sac is produced through the ·local development of muscle. These muscles move the parts of the animal directly and not indirectly as in an animal with a hard skeleton. The complex series of antagonistic muscles is not required to move the part. Retraction of any part is produced by the contraction of one muscle, and extension is due to relaxation under the influence of the hydrostatic pressure within the body cavity. Each simple response of the animal tends to be the result of the action of a single muscle, or group of synergic muscles. And the more complex responses can fairly easily be analysed into such constituent simple responses. The musculature of the Actinozoa differs considerably in different species. That of *Calliactis* resembles fairly closely the system described by Parker and Titus (1916) in *Metridium*, which is closely related to *Calliactis* though these two animals differ significantly in certain features. Hertwig and Hertwig (1879) described the musculature of *Calliactis* (*Sagartia*) *parasitica*, but the relation of the particular muscles to their function was not considered. The following account deals with the muscles involved in the various responses. The musculature is illustrated in Fig. 1.

MUSCULATURE OF THE COLUMN.

The only muscle layer in the column itself appears to be the circular fibres of endodermal origin. Over the greater part of the column this consists of a thin layer of fibres, but in the upper quarter where the column joins the disc the circular muscle has become elaborated into a highly complex and specialised marginal sphincter. This is a mesogloeal sphincter. Its muscle fibres are large and specialised, and in vertical section the sphincter is seen to possess a fairly well-defined limit confining it to the upper part of the mesogloea.

The general layer of circular muscle and the specialised marginal sphincter constitute two muscular systems which are functionally absolutely distinct. It is the contraction of the sphincter which covers the disc when the anemone is irritated.
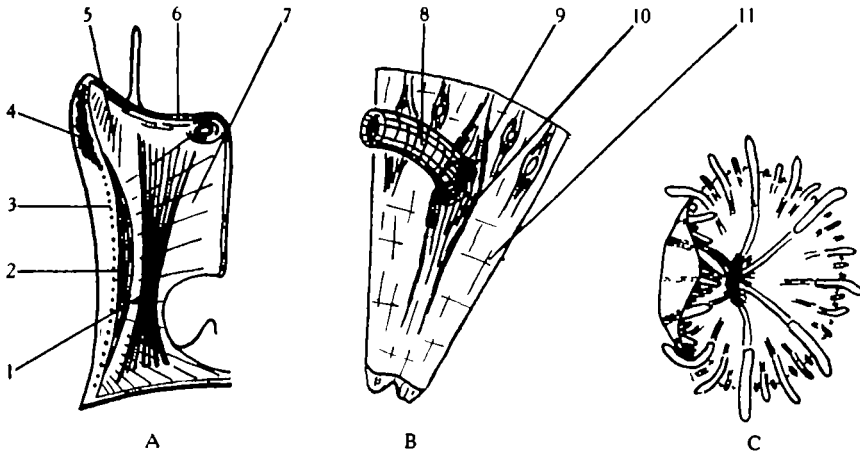

Fig. 1. A. Partial cross-section of *Calliactis parasitica* to show muscles of a complete mesentery: 1, longitudinal mesenteric; 2, parietal; 3, circular muscle of column; 4, marginal sphincter; 5, marginal mesenterics; 6, radial mesenterics; 7, transverse mesenterics. B. Sector of disc: 8, tentacle; 9, radial mesenterics; 10, radial extensions of longitudinal tentaculars; 11, radial and circular muscles of disc. C. Disc, showing well-developed "edge-raising" reaction.

The response produced by contraction of the general circular layer is not concerned in this and will be considered later on. There is no evidence of any longitudinal muscle in the column.

MESENTERIC MUSCULATURE.

The numerous mesenteries of *Calliactis* provide the animal with its longitudinal musculature. In the primary mesenteries, and indeed to some extent in all the older mesenteries, there are well-developed longitudinal mesenteric fibres running from the disc to the foot, and their contraction is responsible for the lowering of the disc. In transverse section, these longitudinal mesenteric muscles appear as well-developed "diffuse" muscles on the side of the mesenteries.

In addition to this system there exist on either side of the very base of all the mesenteries, young or old, well-developed parietal muscles, which are responsible

for the retraction of the column as opposed to the disc. Occasional transverse fibres occur throughout the length of the mesentery. Just beneath the attachment of the mesentery to the disc these form a fairly well-developed band of radial mesenteric fibres.

DISC MUSCULATURE.

The surface of the disc is divided radially into sectors of greater or less extent by the various mesenteries. The whole disc is pervaded to some extent with radial and circular fibres. The radial mesenteric muscles also affect the contractions of the disc.

The tentacles consist of the usual longitudinal and circular fibres; but the longitudinal fibres extend into the disc itself for a considerable distance, and function with the tentacle as a single unit. This radial-tentacular musculature is particularly well developed on that part of the intermesenteric space towards the mouth.

THE RESPONSES TO MECHANICAL STIMULATION.

Stimulation of column.

If the column is stimulated by means of a glass rod or a silver wire, it is found that the general sensitivity and the nature of the response varies in different parts. Sensitivity is greatest at the pedal edge and decreases as we pass upwards towards the disc. Very light stimulation on the middle of the column, if long continued, tends to cause local contraction of the circular muscle of the column in a ring from the site of excitation. This constriction may slowly pass up the column from the point of excitation as a peristaltic wave. Light stroking of the column may also produce occasional slight upward contractions of individual tentacles scattered round the disc. A somewhat stronger stimulus causes a very slow contraction of the column, so that the disc is lowered though it remains expanded. This is due to the contraction of the parietals. A still stronger stimulus causes, in addition to this effect, a depression of the disc itself, owing to the contraction of the longitudinal mesenteric muscles (Fig. 1). Parker (1916) records similar effects in *Metridium*, where the contraction of these longitudinal muscles appears to be largely responsible for the protection of the disc by invagination. In *Calliactis*, however, these responses are slow and from the point of view of protection of the disc unimportant compared with that which follows great mechanical stimulation. This is a series of violent discrete contractions of the marginal sphincter, so that the disc becomes completely covered before any other reaction of the anemone is apparent. The sphincter reaction is called forth more easily from stimulation of the column than from any other region.

In *Calliactis*, stimulation of the column never causes special responses of the disc. Further, such stimulation evokes only symmetrical responses, that is an even activation of one effector or group of effectors all round the axis of the animal, with no greater activity in the region of stimulation than elsewhere. The reactions to stimulation of the column illustrate the diffuse conduction of excitation from a

stimulus, and the variation in the nature of a response with the strength of stimulus. There is, however, in this case, no evidence that the strength of stimulus affects the distance through which excitation is propagated.

Stimulation of the disc and tentacles.

The most striking difference between the responses of the column and those of the disc and tentacles is found in the variety and asymmetrical character of the latter. A light stimulus on the side of a tentacle causes it to bend owing to the very local contractions of the longitudinal muscles. A prolonged stimulus tends to cause rapid and complete contraction distal from the point of stimulation, though the proximal portion of the tentacle remains unaffected (cf. von Uexküll). Only by repeated stimulation of a tentacle can excitation be caused to spread to other tentacles, and the responses are thus restricted to the sector of the disc including the site of stimulus.

The varied responses of the disc represent various degrees of a feeding reaction. Thus local stimulation of a tentacle as presented in the expanded animal results in the tentacle bending towards the mouth, carrying with it the object which caused the stimulus. If the stimulation is at the base of the tentacle on the side towards the mouth, the whole tentacle may move bodily mouthwards by its base. The effect is curiously local, and does not involve wide contractions of the disc. It appears to be brought about by the radial extensions towards the mouth of the longitudinal muscles of the tentacle. Continued light stimulation causes these effects to spread to adjacent tentacles.

In many individuals there is a marked local response of the disc itself. In this the edge of the disc is raised at the site of the stimulus above the level of the disc-surface, and over towards the mouth, so that the tentacles on this part of the disc are curved bodily inwards. This "edge-raising" reaction was first described by Nagel (1894). He pointed out that it is at first a purely local contraction. It cannot certainly be said which muscles are involved, but repeated observation of the movement of the disc when this reaction takes place and examination of fixed sections of contracted individuals lead me to believe that the muscles responsible for this are the radial muscles of the mesenteries where these join the disc (Fig. 1). Continued stimulation causes this raising of the edge to extend progressively sector by sector round the disc; though it can rarely be made to involve more than about one-third of the disc before the entirely distinct sphincter contraction is called up; and this rapidly and completely closes the whole surface of the disc symmetrically. In the complete feeding reaction this edge-raising is accompanied by a sinking of the disc between the site of stimulus and the mouth, which is thus drawn towards the stimulating object and may engulf it.

For these responses of the disc an increase of the stimulus may not only vary the response, but apparently increase the distance to which excitation is propagated. As opposed to the symmetrical responses of the column there appears to be some kind of "decrement" in the propagation of excitation of the disc. This same character endows the tentacles and separate parts of the disc with a considerable degree of

autonomy. However, strong and prolonged stimulation finally involves excitation of the symmetrically responding muscles of the column. This occurs much more readily in some individuals than in others. The contraction of the marginal sphincter may be preceded by mesenteric contractions, as after stimulation of the column.

The sensitivity within the disc varies considerably. It is less near the mouth than towards the edge of the disc. Further, the contractions of the marginal sphincter are more easily obtained from the outer tentacles than from the inner ones. The sensitivity of the individual varies considerably with physiological conditions, such as starvation, also the amount of previous stimulation. In particular, frequent application of moderate or weak stimuli produces a condition of local insensitivity. This is peculiarly marked in *Calliactis*.

RESPONSE TO ELECTRICAL STIMULATION.

Method.

The stimulation apparatus was based upon the neon lamp relaxation oscillator (Briscoe and Leyshon, 1930). The discharge circuit usually consisted of a $0.4\,\mu\mathrm{F}$ condenser discharging through a resistance of about 5000 ohms in series with a
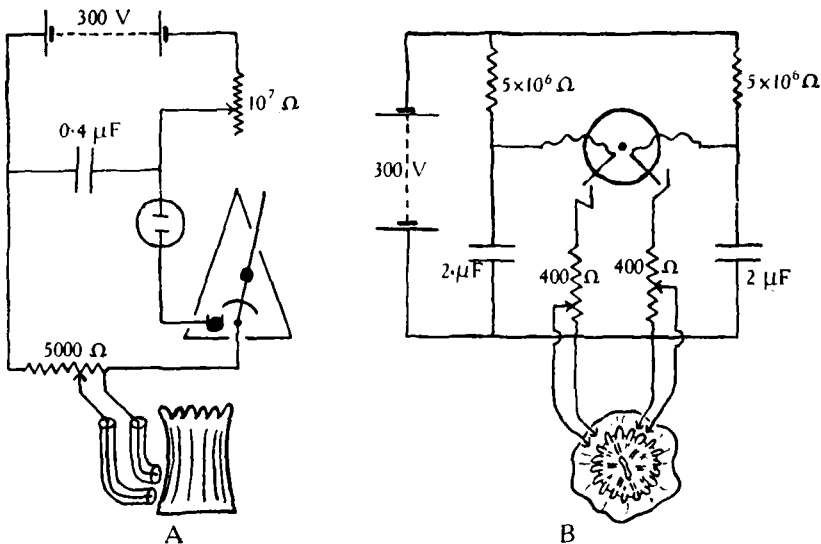

Fig. 2. Details in text. A. Mechanically controlled neon lamp circuit.
B. Refractory period circuit.

neon lamp. The condenser was charged by a 300-volt battery through a variable resistance of about 10 megohms. The 5000-ohm resistance included a potentiometer of a few hundred ohms, from which came leads to the electrodes. These usually consisted of silver wires fixed in narrow glass tubes with sealing wax, the ends being planed off to give a silver surface of about 1 sq. mm. surrounded by a flat area of sealing wax. Where fully non-polarisable electrodes had to be employed, these were

constructed of zinc—zinc sulphate—agar plus sea water, a 2 mm. hole being blown in the bottom of a test-tube to allow conduction of the current from the electrode to the organism.

In most experiments it was desirable to record graphically the exact moment at which each stimulus was given. This was done in the following way. A metronome was placed in the condenser discharge circuit. The natural frequency of the discharge was then adjusted to be about 5 per cent. in excess of the frequency of the metronome. The metronome by making a mercury contact was then allowed to discharge the condenser at its own frequency. With this mechanically controlled arrangement, it is an easy matter to provide a simultaneous record of the moment of discharge by a separate contact in a high-speed time marker circuit (Fig. 2). It was found most convenient to record the contractions by means of very weak isotonic levers.

Responses of the column.

The responses evoked by electrical stimulation of the column exactly resemble those obtained by mechanical stimulation except that the local fatigue is much less evident. The contractions of the marginal sphincter are easily recorded graphically. The sphincter contracts so rapidly and powerfully that there is no danger of its response being confused with that of other muscles. Stimuli of a frequency 1 per sec. evoke excellent discrete contractions in the sphincter which show a well-marked "staircase" (Figs. 3 and 4).

In view of the supposed importance of the strength of stimulus it is of particular interest to determine its relation to the response when electric stimuli are employed. If with an electrical stimulus of constant frequency the intensity is gradually raised a well-defined threshold of excitation is found. Above this, good contractions abruptly appear, and further increase in the strength of the stimulus in no way increases or modifies their response. The effect is striking. Fig. 3 shows the complete independence in the response of the sphincter over a 15-fold increase in current intensity. It would seem scarcely possible to obtain a clearer demonstration of that independence between strength of stimulus and response so characteristic of ordinary nerve than is shown in this way by the nerve net. Although the response does not vary with the strength of the stimuli, it is very sensitive to their number and time relations. Indeed, a single stimulus has no visible effect. It is only when it is followed by another that a contraction takes place; and the size of this depends upon the time interval between the two stimuli. Fig. 4 shows the responses of the marginal sphincter to increasing frequencies of stimulation. The rate of contraction increases greatly. This is not merely due to mechanical summation of the more frequent individual contractions. The magnitude of these contractions themselves progressively increases with the frequency. The response is quite evidently a simple and striking *facilitation* phenomenon. That is the excitation wave from each stimulus not only activates some of the muscle-fibres, but leaves behind it an after-effect which gradually subsides. This after-effect facilitates the transmission of the next excitation wave to the muscle, so that yet more muscle fibres are activated.
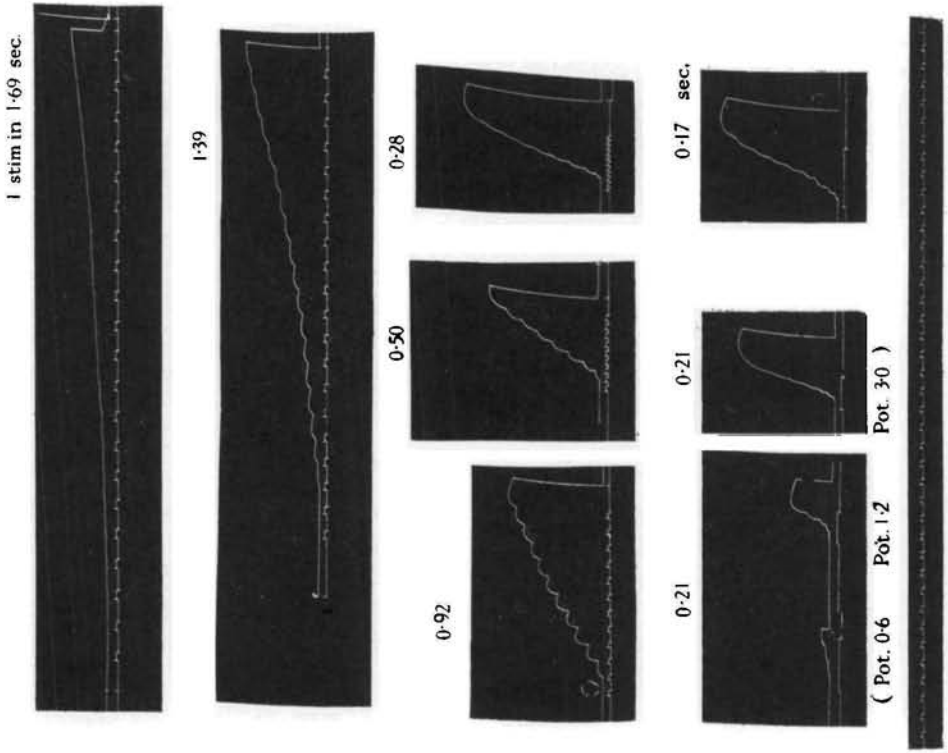
Fig. 4. Sphincter. Relation of response to frequency.
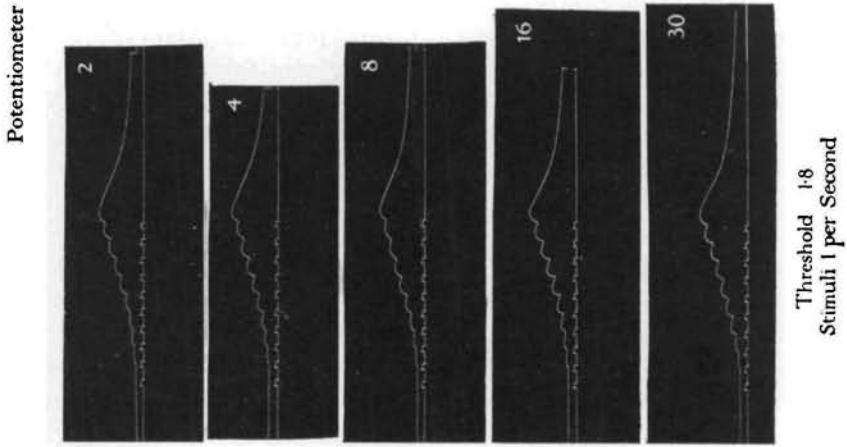

Threshold 1·8
Stimuli 1 per Second

Fig. 3. Sphincter. Relation of response to strength of stimulus.

The importance of frequency and the independence from intensity is found for the responses of the mesenteric and parietal as well as the sphincter. The responses can be produced from any point on the column.

These experiments show conclusively, first, that when a response occurs it is the result of many stimuli, and not of a single stimulus. Secondly, that the nature of the response depends upon the number of stimuli and upon the interval of time between them. Thirdly, that the strength of the individual stimulus is without influence. This raises the question how we can interpret the usual statement that the response varies with the "strength" of the stimulus. There seems no doubt that it is due to the employment of mechanical and similar methods of stimulation. A mechanical stimulus acts upon one or more sense organs, and from these not one but a number of nervous impulses are sent out. The greater the "strength" of such a stimulus, the greater the number and frequency of discharges from the stimulated sense
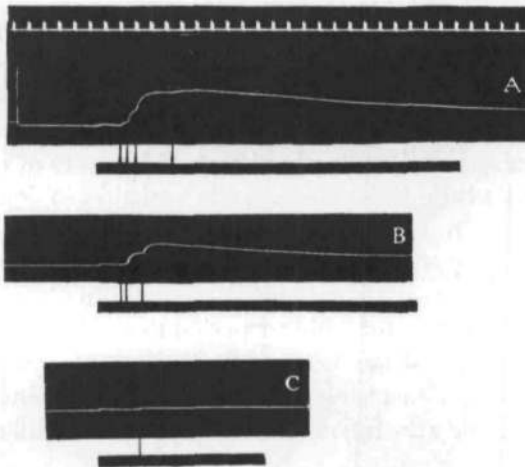

Fig. 5. A, B and C. Graded responses of sphincter to mechanical stimulation of pedal edge. Black ribbon = duration of mechanical stimulus. Vertical lines show arrival of impulses at sphincter. First line corresponds to arrival of second impulse: first impulse causes no response. Note adaptation.

organ. That mechanical stimulation does give rise to a battery of impulses is shown by Fig. 5. This illustrates the response of *Calliactis* to mechanical stimulation of the pedal edge. A weighted point is allowed to fall upon it at the moment shown in the time signal. There is a series of discrete contractions. By noting the moments at which each contraction takes place it is possible to follow the succession of impulses discharged by the sense organs involved. It will be seen that the time interval between the discharges increases with the time that has elapsed from the beginning of the mechanical stimulus. Indeed, this response gives a direct demonstration of sensory adaptation (Adrian, 1932). Fig. 5 also shows that as in electric stimulation the size of the contraction depends upon the time interval between the impulses set up. At the beginning of the mechanical stimulus the impulses are close together, and the response is greater than when the impulses become less frequent and facilitation falls off.

For these reasons, any statement with regard to the relationship between response and strength of stimulus must be very carefully considered. A strong mechanical stimulus involving sense organs is not comparable to a single electric stimulus applied to a nerve. Hence there is no reason to suppose that the relation of response to stimulus in the nerve net necessitates properties which are not in full accordance with what we already know of conduction in nerve.

Responses of the disc region.

The responses of the disc differ from those of the column in showing autonomy, greater variety, and apparent decremental conduction. The question arises, how far is it possible to interpret these in terms of the conclusions at which we have just arrived? Electrical stimulation of the disc presents certain difficulties. It is much harder to place electrodes on it without involving tactile stimuli than on the column, where sensory adaptation is rapid (cf. Fig. 5). Also slight movements of the disc are apt to alter the effective threshold of excitation. It is also possible that, owing to the autonomy of the various sectors of the disc, an intense stimulus may independently excite separate regions. These difficulties can be partly overcome by careful isolation of the part stimulated, and in spite of their existence definite evidence can be obtained to show that variation of intensity of the stimulus at any one site is of relatively little importance, while variation of frequency profoundly modifies the response. In Table I is recorded the response of various animals to stimuli applied to the disc at the base of the tentacles. The frequency and intensity of the stimulus is given. There is a tendency for stimuli of a higher intensity to continue to produce responses for a longer period than stimuli of a lower intensity. But, on the other hand, at all intensities a low frequency restricts the response to types characteristic of weak mechanical stimulation, whereas increase in frequency of stimulus promptly evokes the full range of active responses whatever the intensity,

Table I.

o = no response.
a = local contraction of one tentacle.
b = movement of a few tentacles all round disc.
$c-c'''$ = local raising of edge of disc of increasing extent, with movement of local tentacles.
d = slow contraction of column.
$e-e'''$ = increasing stages of sphincter contraction.
x = complete contraction of whole anemone.

Potentiometer	Frequency	Response to successive stimuli
15	1 in 4 sec.	$o, a, a, b, b, b, b, b, d, o$
30	1 in 4 sec.	$o, a, b, b, b, b, b, b, b, b, b, e, b, b, b, o, a, a, a, o$
30	1 in 0·74 sec.	o, a, b, x
15	1 in 0·74 sec.	o, a, b, x
10	1 in 10 sec.	o, c, o, c, c, o, o
30	1 in 10 sec.	o, c, c, c, o, o, o
6	1 in 2·5 sec.	$o, c, c, c, c, c, c, c', c'', c'''$
30	1 in 2·5 sec.	$o, c, c, c, c, c, c, c, c, c', c'', c'''$
30	1 in 1·0 sec.	o, c', e, e, e', e'', x
5	1 in 4 sec. then 1 in 1·4 sec.	$o, a, b, b, b, b, b, b, b, b$ e, e', e'', x

so long as this is above the threshold value. This is very clear when a change in frequency is made during a succession of stimuli, and a series of minor responses is abruptly converted into the most active ones, as in Table I.

In the disc, therefore, a single stimulus produces only a slight response or none at all. All the more complex responses require a battery of stimuli, and it is the time interval between stimuli which governs the nature of the response, and not their strength. It seems reasonable to suppose, therefore, that the various responses of the disc are called up in succession through facilitation.

The second special feature of the disc responses is the apparent existence of a decrement of conduction. This is apparent in the "edge-raising" reaction. The stronger the mechanical stimulus, the more widely is the reaction propagated round the disc. The reaction is easily evoked electrically, but only by a battery of several stimuli. Here again above the threshold, the strength of the stimulus is of no importance; but as the frequency of the stimuli in the battery increases there is a great increase in the size of the response and also it is propagated further. Its propagation is easily studied with low-frequency stimuli. There is not a single contraction wave propagated outwards with an intensity that decreases as it gets further from the point of stimulation; though this might have been expected were the conducted excitation to undergo a decrement. Actually each stimulus of the series calls up a contraction, but with each successive stimulus the contraction extends by stages further and further round the disc, and ceases to extend the moment the battery of stimuli comes to an end. While, therefore, the muscles under the electrodes respond with contractions to the first few stimuli, those some distance away round the disc do not begin to respond till several stimuli have already passed, after which they respond in a normal manner. One cannot interpret these effects by supposing that a stimulus is conducted with a decrement. Evidently, in a battery of successive stimuli, each member paves the way for the propagation of its successors into fresh sectors of the disc. The most simple explanation of this is that the conducting path extends by progressive facilitation between the conducting units of the adjoining sectors; that is, the response of a sector on the outskirts of the contracting area differs from the response immediately under the site of the electrodes only in that several stimuli are required before a conducting path is established to it. It will not therefore begin to respond to each stimulus until several stimuli have taken place. This implies that facilitation is taking place between different sections of the nerve net itself. This "interneural" facilitation will be considered again in a later paper.

This interpretation of decremental conduction may be extended to other cases. Bozler (1926) has pointed out that decrementless conduction must obtain in certain tracts of the nerve net of Medusae. But there are other tracts in which he discovers a strong decrement, and this he correlates with the appearance of synapse-like properties between the conducting units. These tracts in many ways resemble the disc nerve net in their properties. It seems likely that, as in the disc, the apparent decrement is associated with the response to a battery of many nervous impulses the spread of which to adjacent conducting units depends on facilitation.

This same interpretation can apply to the nerve net of Echinoids. Both *Strongylocentrotus lividus* and *Arbacia pustulata* show strong conduction decrements as measured by the response of the spines to mechanical stimulation. A number of experiments was performed on these, using electrical stimulation. With stimuli varying in frequency from 1 in 4 sec. to 1 in 0·3 sec., both the extent of the movement of each spine towards the electrodes and the distance to which excitation is propagated increase very rapidly with the frequency of excitation and the number of stimuli given. Variation of the intensity of stimulus, on the other hand, produces but little effect.

There is in fact no good evidence for the existence of a conduction decrement at all, except perhaps in the totally new sense that in such regions of the nerve net a battery of impulses may suffer a decrement of number as it passes into adjacent conducting units, though not of individual intensity. But the use of the term "decrement" in this case is so far removed from its accepted use that it is better not to employ it. In any case, a "decrement" in the number of successfully conducted stimuli passing from one part of the conducting system to another gives no ground for the supposition that the nature of conduction in the nerve net differs from that in ordinary nerve. It is therefore valid to use the hypothesis that the nerve net possesses such properties, and that its peculiar features are due to the special importance of facilitation.

PHYSIOLOGICAL PROPERTIES OF THE NERVE NET.

The peculiar properties that have been ascribed to the nerve net make it necessary to determine how far it is physiologically similar to ordinary nerve. Inherent properties of nerve such as refractory period have been frequently assumed to exist in the nerve net but the evidence is unsatisfactory, and these properties seem to be incongruous with many of its apparent features.

Chronaxie.

If the nerve net is physiologically similar to nerve, we would expect the appearance of the familiar relationship between the intensity of a stimulating current and the duration necessary for it to cause excitation. The contractions of the marginal sphincter give a splendidly critical indication of the success of excitation, but a succession of stimuli is necessary for a response. The periodic condenser discharges of a neon lamp oscillator provide a simple means of simultaneously controlling the frequency, strength and duration of a series of stimuli.

The threshold intensity for excitation at each capacity can be found at any desired frequency of stimulation. The duration of the discharge is approximately proportional to the capacity of the condenser over a fairly wide range. In these experiments this was directly determined by estimating the duration of the flash of light in the neon lamp which takes place at each discharge. This was done by means of a revolving mirror. An approximate scale of discharge durations for the particular lamp and circuit employed was thus constructed and placed alongside a scale of capacities, as in Fig. 6.

In the circuit generally used, the condenser was charged by a 300-volt battery through a 10-megohm variable resistance. The resistance in the discharge circuit, including a small potentiometer from which arose the stimulating electrodes, was about 400 ohms. The resistance of the non-polarisable electrodes in contact with the anemone was usually about 200–300 ohms. The electrodes were placed on the lower half of the column of the anemone, and the frequency of stimulation was adjusted to 1 per sec. The method works well over an enormous range of frequencies. In unpublished experiments on crustacean nerve comparison of intensity duration curves determined by the neon lamp method with those obtained by means of a Lucas spring rheotome showed that only about 0·5 of the duration of the discharge of the neon lamp was effective for excitation. The chronaxie therefore probably
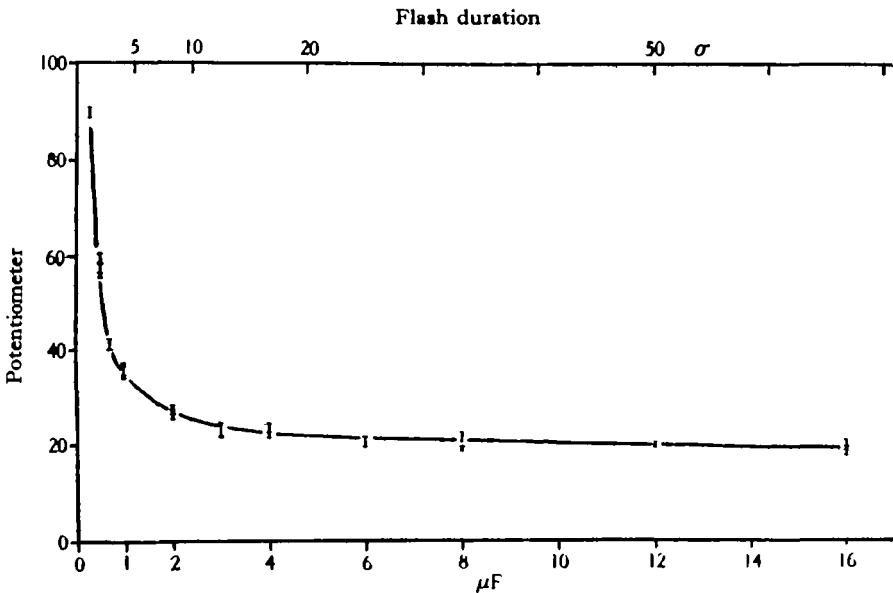

Fig. 6. Intensity duration relation for column nerve net.

corresponds to about 0·5 time duration of the condenser discharge when the threshold intensity is double rheobase strength.

The result of a typical experiment is shown in Fig. 6. It shows that the actinozoan nerve net can yield an intensity duration curve of the usual form. To estimate a chronaxie, a rheobase must be determined for currents of very long duration. In the present case, this is done by using condensers of very great capacity. To approach constancy of threshold in the nerve-net very long stimuli are required. This condition is fulfilled in Fig. 6, but it is often difficult to determine the rheobase because repeated application of currents of long duration may produce local insensitivity. The sudden development of local insensitivity after repeated stimulation was fairly often met with. A small change in the position of the electrodes or even a reversal of their sign allows excitation to take place again.

In Fig. 6 the duration of a condenser discharge which succeeds in exciting at double rheobase strength is 5–6σ. Comparison of many such experiments shows that this value falls between 4 and 7σ. Applying the factor 0·5 to this, the chronaxie appears to fall between the limits 2–4σ. This value may be compared with a chronaxie of 2σ for vaso-constrictor fibres in the frog. The excitability of the nerve net thus shows no special features which would lead us to distinguish it from nerve. Indeed the resemblance is closer than might have been expected. Considering the slow responses of the anemone and considering that stimulation at a frequency of only 1 per sec. is sufficient to evoke the most active responses of the animal, the chronaxie is surprisingly small.

Refractory period.

The refractory period of the nerve net was determined as follows. Two pairs of electrodes, each 2 mm. apart, were placed against the base of the column of the anemone at a distance of about 4 cm. from each other. The vertical distance from each electrode to the marginal sphincter was the same. A condenser discharge was then passed through each pair of electrodes in succession. The intensity of each discharge and the interval between them was varied as desired. Fig. 2 shows the usual arrangement. A battery of 300 volts slowly charges two 2 μF condensers through 5-megohm resistances. A clockwork turn-table carrying contact arms successively discharges the two condensers each through its own resistance of 400 ohms, which includes a 30-ohm potentiometer. The minimal intensity required on each potentiometer is first found by discharging first with one condenser leading and then with the other at an interval of 1 sec. Having done this, the intensity from the leading condenser is maintained just in excess of the threshold value, while the strength required to give a facilitated response following the discharge of the second condenser is determined for various time intervals. Fig. 7 shows a curve obtained in this way. There is a well-defined absolute and a long relative refractory period. The threshold has returned to normal about 0·5 sec. after the first stimulus. The absolute refractory period in Fig. 7 is about 60σ, a typical value. In different experiments this varied somewhat, the lowest value being about 40σ.

The true value of the absolute refractory period is hard to ascertain. In a simple nerve trunk, only one path is possible for all impulses: in the nerve network alternative paths are possible. In the present experiment, if excitation is conducted from each pair of electrodes directly up to the sphincter, the paths traversed are of equal length. Any delay between stimulus and response due to conduction would be the same for each electrode pair. The absolute refractory period as measured would then be a true one. It will be shown later that conduction in the nerve net of the column does closely approach this condition. But small inequalities of path may affect the apparent value of the absolute refractory period owing to the comparative slowness of conduction in the nerve net. Such errors, however, cannot be very large, because similar values are obtained for the refractory period with either condenser leading. In general, the absolute refractory period appears to lie between 40 and 65σ.

The refractory period sets a limit to the frequency of effective stimulation of the nerve net. When a battery of stimuli is applied to the column a facilitated response is produced as in Fig. 4. The threshold remains the same until the stimulation interval becomes less than o·5 sec. As we see from Fig. 7, this corresponds to the beginning of the relative refractory period. If several stimuli are sent in at intervals within the relative refractory period, the threshold appears to rise steadily. Thus, in Fig. 4, three series of contractions are shown at a stimulation interval of o·21 sec. Just above threshold intensity, the sphincter responds only to every third stimulus,
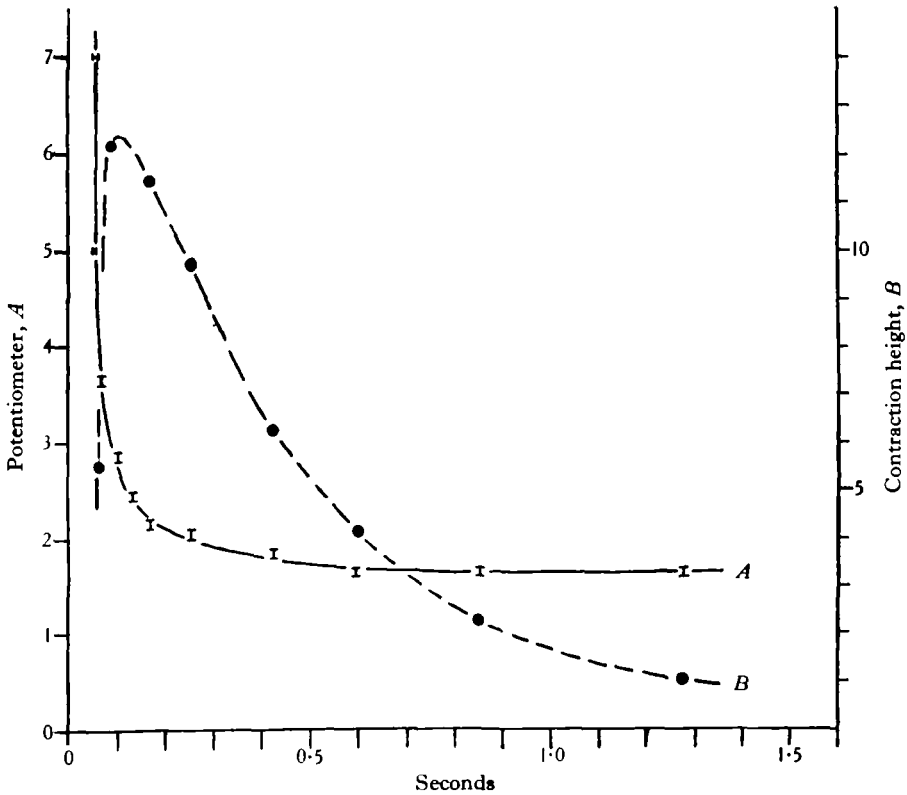

Fig. 7. Refractory period and facilitation of sphincter.

at double this intensity to every other stimulus, while the intensity has to be raised to five times the threshold value to ensure a response to every stimulus. For a stimulation interval of o·21 sec. every stimulus falls well within the relative refractory period of its predecessor, and even every second stimulus will be not quite clear of it. A comparison of the above threshold values with Fig. 7 shows that the threshold rises much more rapidly when a battery of more than two stimuli are sent in at intervals within the relative refractory period. It is as though the absolute refractory period was considerably lengthened with increasing numbers of stimuli. But it is hard to be certain of this, because repeated stimulation at intervals within the

refractory period not only temporarily raises the threshold but tends to induce complete local insensitivity which may be maintained for some time.

The absolute refractory period for two stimuli would correspond to a limiting frequency of about 20 per sec. The natural rate of response does not seem to reach more than half this at most.

The mode of facilitation.

By recording the response of the sphincter with a weak isometric lever, the same experiments on which refractory period is determined serve to estimate the relationship of facilitation to the interval between stimuli. No response takes place to the first stimulus. The contraction height is thus a statistical measure of the success of the second stimulus in reaching the muscle fibres. It is shown in Fig. 7.

Facilitation becomes evident when the stimulation interval far exceeds the refractory period. As the interval is shortened, the facilitated response increases more and more rapidly, right into the relative refractory period. It reaches a maximum, however, between 0·2 and 0·1 sec., and below this the height of contraction falls off sharply, even though there is no question that the second stimulus is effective. The cause of this effect may be that when the stimulation interval is so short all the branches of the nerve net in contact with the sphincter muscle fibres may not have emerged from the absolute refractory period.

It is interesting to notice that facilitation commences long before the refractory period is entered and reaches a maximum actually in the relative refractory period itself. There is no supernormal phase; so that facilitation cannot be related to this, after the manner suggested by Keith Lucas (1917) for crustacean muscle. On the other hand, the results bear a definite resemblance to those obtained by Bremer on the partially curarised sciatic-gastrocnemius of the frog (Bremer, F., 1930), and may agree with his hypothesis that there is an actual summation of the effects of the action current.

DISCUSSION.

In the description of the foregoing experiments it was assumed that electrical stimuli directly excite the nerve net, and further that the physiological properties which have been investigated, such as the refractory period, are characteristic of the nerve net and of no other part of the excitable system. But the experiments have involved the stimulation of an entire organism, and caution must be exercised in comparing them with the results of stimulation of an isolated nerve trunk attached to a muscle. The whole excitable system consists of sense organs, the nervous units which constitute the nerve net and the muscles, and we must consider which of these actually responds to stimulation. It is certain that the muscle itself is not directly stimulated. The electrodes are situated at a considerable distance from the sphincter, and it is easy to show that the response of the latter depends upon the integrity of the intervening tissues of the animal. On the other hand, the response to an electrical stimulus differs materially from that following direct stimulation of a sense organ, for that is followed by the discharge of a battery of impulses which

comes to an end while these organs undergo their characteristically rapid adaptation whereas each electrical stimulus only produces a single response.

A great deal of light can be thrown on the problem by a study of the refractory period. Since it is obtained by successive stimulation at two independent sites, the refractory period cannot be that of separate localised excitable elements which transmit excitation to the nerve net. For the refractory period of an element restricted to the neighbourhood of the first electrode cannot affect the success of a stimulus applied to a similar element elsewhere. The refractory period must therefore apply either to the nerve net itself, or to the responding muscle. But, from Fig. 7, stimuli applied to two independent sites on the column show the existence of a relative refractory period with a continuously increasing threshold up to the absolute refractory period. This can only be explained by supposing that the excitation wave following the first stimulus reaches the very region itself of the excitable system which is influenced by the second stimulus. We have seen that the muscle itself is not directly excited, there remains therefore the only possibility that the stimuli are directly exciting the nerve net, and that the refractory period measured is that of some part of the nerve net itself. It does not necessarily follow that the refractory period of all parts of the nerve net is the same, but the stimuli excite a physiologically continuous system, and the measured refractory period applies to those parts of the system immediately below the stimulating electrodes.

The evidence also shows that the wave of excitation passes over the entire nerve net of the column, for stimulation at any point on it throws the whole column into a refractory period. In addition to this there is a mass of evidence to show that the nerve net of the column not only conducts excitation diffusely in all directions, but behaves as a single conducting unit. In the response of the sphincter, although there is no response to the first stimulus, a conducting path is certainly established through from the site of stimulation to the muscle by the second. Again, in responsive animals, a single stimulus may produce a reaction in the form of a slight waving of tentacles scattered round the disc. This reaction is evoked from any point on the column and requires no previous stimulus to ensure its appearance. We are thus brought to the remarkable conclusion that the whole nerve net of the column acts as a conducting layer in its most simple form, directly transmitting excitation from the site of stimulus to the muscle. Such an arrangement is far simpler than the most elementary reflex arc, and indeed it is simpler than anything which the nerve net has previously been considered to exhibit. This simplicity is restricted to the nerve net of the column and does not obtain in that of the disc. It is the cause of the symmetrical character of the responses to stimulation of the column. The contraction of the sphincter, of the parietal, or of the longitudinal mesenteric muscles does not develop gradually from the region of the stimulus. The muscles are activated equally all round the axis of the animal. But with so simple a conducting mechanism, the question naturally arises: how can any variety of response, such as undoubtedly follows stimulation of the column be obtained? We shall consider this fully in a subsequent paper. But since the nerve net of the column behaves as a single conducting unit, it is evident that the facilitation found in the response of such

muscles as the sphincter must occur between the nerve net and each individual muscle, and it is evident that there is scope here for a considerable variety of response.

In conclusion, let us consider the special properties of the nerve net in the light of the results obtained. Of diffuse excitation there is no doubt, though it is clear that it may be of two kinds, complete, as in the nerve net of the column, or restricted, as in conduction in the disc. But the statement that the response varies with the strength of the stimulus needs complete reconsideration. An excitation wave set up by stimulation of the nerve net is independent of the strength of stimulus. In actual fact, a stimulus which succeeds in producing only a single excitation wave in the nerve net produces no response at all or only a trivial one, whatever may be its strength. But a stimulus applied to the surface of the animal excites the sense organs to send out not one, but a group of impulses and it is entirely upon the number of, and interval between these, that the response depends. Finally, the supposed conduction of excitation with a decrement is not true under any circumstances. The distance through which a single excitation wave is propagated is independent of the strength of the stimulus. In the column nerve net of *Calliactis* the excitation wave passes over the whole system. In the nerve net of the disc, on the other hand, the excitation wave is restricted to the region stimulated. Excitation, however, is propagated to adjoining regions when several stimuli succeed each other at short intervals. Though there is no evidence of a decrement in the intensity of an individual excitation wave, the distance excitation is propagated in the disc increases with the number and frequency of the excitation waves set up. By facilitation between adjacent parts of the nerve net, each successive excitation wave is enabled to travel further than the last. But the stronger a mechanical stimulus on the disc, the greater is the number and frequency of impulses discharged from the sense organs; and hence the further does the response spread.

The nerve net consists of units which behave like true nerve. It is characteristic that it tends to conduct stimuli in all directions; but its most striking feature is the extreme degree to which facilitation is developed both within the net and between the net and the muscles.

SUMMARY.

1. Certain features have been supposed to characterise the nerve net. Response is said to vary with the strength of stimulus: and while conduction may occasionally take place in an all or nothing manner, yet in general conduction is supposed to take place with a decrement. To investigate these points, the responses of *Calliactis parasitica* to mechanical and electrical stimuli have been investigated.

2. Electrical excitation of the column of the anemone shows that a response results from a succession of stimuli and not from a single stimulus. The character of the response is independent of the strength of the individual stimuli. It depends solely upon the number of stimuli and upon the interval of time between them. All responses are highly developed facilitation phenomena. Each electrical stimulus induces a single excitation impulse in the nerve net.

3. But a mechanical stimulus is followed by the discharge of a battery of impulses from the sense organs. These increase in number and frequency with the intensity of the mechanical stimulus. A response may therefore vary with the strength of a mechanical stimulus, but only in relation to the number and frequency of impulses discharged by the sense-organs.

4. Stimulation of the intact column of the anemone shows complete conduction over its whole nerve net. Mechanical stimulation of the disc appears to show conduction with a decrement. Such a stimulus excites a battery of impulses. Each impulse is conducted without decrement, but it facilitates the entrance of succeeding impulses into adjoining sections of the disc nerve net.

There is no decrement of excitation strength under any conditions in the nerve net. But there may be a numerical decrement as a battery of impulses spreads from a stimulus.

5. The nerve net is physiologically similar to ordinary nerve. A method is described for determining the strength duration relationship for the threshold of electrical excitation of the nerve net. The relation is of the usual form with a chronaxie of about 2–4σ. The nerve net has well-defined relative and absolute refractory periods: the latter is about 40–65σ. The relation of facilitation to refractory period is considered.

6. The whole nerve net of the column of the anemone acts as a conducting layer in its most simple form, directly transmitting excitation from the stimulus to the muscle. Facilitation takes place between the nerve net and its appropriate muscles. Conduction in the disc nerve net involves facilitation between parts of the nerve net in addition.

7. The true characteristics of the nerve net are diffuse conduction and the extreme development of facilitation. Diffuse conduction may be total, as in the column, or restricted, as in the disc of *Calliactis*. Facilitation may be between the nerve net and the muscles, or between parts of the nerve net.

138 C. F. A. PANTIN

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