THE NERVE NET OF THE ACTINOZOA

IV. FACILITATION AND THE “STAIRCASE”

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(With Two Text-figures.)

INTRODUCTION.

The responses of the anemone Calliactis parasitica show a variety of summation phenomena. Apart from mechanical summation of contractions in the muscle, two kinds of summation of excitation can be distinguished (Pantin, 1935a). There is a kind of summation between one part of the nerve net and another, and again between the nerve net itself and the muscles. The present paper will deal with this second kind of summation. This is very clearly shown in the response of the marginal sphincter of the disc of the anemone to stimulation of the column. If a succession of stimuli is given, the first stimulus produces no response, while each succeeding one produces a step-like contraction of increasing height (cf. Fig. 2). For reasons given in the earlier paper, it was suggested that this effect was due to facilitation at the neuromuscular junction. Thus the first stimulus causes a wave of excitation to travel over the nerve net which, though failing to cause contraction, yet leaves behind it an effect which facilitates the passage of the second impulse to the muscle fibres. The excitation wave from each subsequent stimulus then not only activates these muscle fibres but facilitates the transmission of subsequent excitation waves to yet more muscle fibres. The effect was referred to as “neuromuscular facilitation”, and the essential feature of this hypothesis is that the increment of contraction from each shock is due primarily to successive increase in the numbers of muscle fibres responding.

In a recent paper Bethe (1935) has extended his earlier work upon Medusae. He traces a far-reaching parallelism between their rhythmic contractions and those of the vertebrate heart. In some respects this parallel is significant, particularly with regard to the great length of the refractory period relative to the duration of the muscular contraction. There are, however, certain other analogies where the parallelism requires consideration, particularly the marked “staircase” shown by both the resting vertebrate heart and the Medusa bell. In both cases, if the “pace-maker” has been removed and the system allowed a long period of rest, the responses to the first of a succession of stimuli is much below the normal size but increases with each successive stimulus.
The staircase in the vertebrate heart is peculiar. The heart is a syncytium and, as the classic experiments of Bowditch (1871) showed, it contracts in an all-or-nothing manner. Mines (1913) showed that in all stages in the staircase all the muscle fibres were involved, since the strongest induction shocks did not increase the height of contraction in the first step of a staircase in the resting ventricle. Hence the increasing contractions of the staircase were due to a modification of the state of the muscle fibres, which rendered their powers of contraction more effective. Mines devised an hypothesis to show how this effect could be brought about through the accumulation of products of contraction such as lactic acid. This was later shown to be wrong (Adrian, 1920), but there is strong evidence that the effect is a cumulative result of contractions, probably through the accumulation of metabolites other than lactic acid (Dale, 1932).

A complete analogy between the vertebrate heart and the Medusa implies that the neuromuscular system of the latter behaves as a single conducting unit; that all the muscle fibres are activated with each stimulus; and that the staircase phenomenon is due to increasing efficiency of action of the muscle fibres, rather than to an increase in the number of those contracting. There are, however, certain difficulties in accepting this. The staircase in a Medusa is very much more complete than in the heart. However long an isolated ventricle remains at rest the first stimulus always produces a contraction, whereas the first steps of the staircase in a Medusa may produce no visible contraction at all. As Romanes (1877) showed long ago in Aurelia this makes it difficult to suppose that the staircase in Medusae depends on the process of contraction. Again, the heart is a single conducting unit, whereas in the Medusa we have a double system of nerve net and muscle cells activated by it. A. G. Mayer (1908) has shown that under the influence of excess magnesium salts excitation may be conducted over the nerve net without causing contraction of the muscle. The two tissues are physiologically separate. Finally the structural organisation of Medusae is closely related to that of other coelenterates such as Calliactis. The step-like contractions of the sphincter of the latter are clearly analogous to the staircase of a Medusa, and just as in the sphincter the staircase might be ascribed to facilitation between nerve net and muscle, this explanation might equally apply to Medusae: but it cannot apply to the vertebrate heart.

We are thus left with two hypotheses. By one, the staircase may be due primarily to successive increases in the numbers of muscle fibres responding to each stimulus. By analogy with the vertebrate heart each stimulus involves the whole muscle and the staircase is due to a progressive change of physiological state by which the contractile efficiency of the muscle as a whole is progressively increased.

NEUROMUSCULAR FACILITATION IN CALLIACTIS.

The experiments described in the first paper of this series were repeated and confirmed. So far as these observations are concerned it is only necessary to draw attention to the following points.

(1) A single stimulus applied to the intact column of the anemone throws all other points on the column into an absolute refractory state. This is followed by a
relative refractory state which endures for some time. During this relative refractory period the threshold for stimulation is found to be raised at all points over the column of the anemone. Therefore the excitation wave from the first stimulus must have traversed the whole net and reached these points. Hence all parts of the column nerve net are in direct effective physiological continuity with one another.

(2) In the resting anemone the first stimulus is ineffective. This statement can be made with considerable assurance, because the disc carries numerous tentacles suspended in the water and provides an unusually delicate indicator for the slightest contraction of the marginal sphincter.

(3) A second stimulus following the first at a suitably short interval causes a contraction. The height of this contraction increases more and more rapidly as the stimulation interval is diminished, almost until the absolute refractory period is reached. It falls off sharply just before this point.

From this it follows that although a first excitation wave produces no contraction in the muscle, yet it must certainly reach the muscle, otherwise the height of contraction following the second stimulus could not be dependent on the interval of time between it and the first.

To explain the dependence of the height of the contraction on the interval between the first and second stimuli we might assume that the first impulse leaves behind it an effect which facilitates the passage of the second impulse from the nerve net into the muscle fibres (or possibly muscle fibrillae). The height of the contraction will then be a statistical measure of the success of the second impulse in terms of the number of fibres excited. Alternatively we might assume that the first excitation wave reaches all the contractile units and there causes a change of physiological state, as in the heart, so that they respond to a second stimulus with contraction.

It is evident at the outset that since, as in the case of Aurelia, the first stimulus produces no visible response, the contraction following the second stimulus cannot be a gross consequence of the chemical and other changes resulting from contraction in the way supposed to account for the staircase in the heart. In the previous paper it was assumed on this evidence that the muscle cells were not in fact excited. It was therefore supposed that a single impulse could not pass the neuromuscular junction. This conclusion does not certainly follow, for it is still conceivable that the first impulse might actually pass into the muscle cells and there produce a change of state owing to some reactions which preceded muscular contraction. The resting muscle might be so far from its optimum state that it was quite unable to contract till this precursor change had been brought about by the first stimulus. That is, the first stimulus may fail to produce a contraction because the muscle cells are not themselves in a suitable state to respond to it. If this is true it should be impossible to obtain a contraction out of the muscle to a single stimulus even if the muscle cells were excited directly instead of by way of the nerve net.

A number of experiments were therefore performed to compare the response of the sphincter to changes in intensity of stimulus, both for indirect excitation of the muscle by application of stimuli to distant parts of the nerve net and to application
of stimuli directly over the sphincter. The method of stimulation was similar to that employed in the previous experiments. A mechanically controlled neon-lamp stimulator was used. A 1 μF. condenser was slowly charged by a 300-volt battery through a high resistance and periodically discharged mechanically through a 400-ohm resistance which included a potentiometer. A neon lamp was in series with the potentiometer so that the discharge of the condenser was cut short, thus giving shocks of a finite duration. The natural frequency of the neon-lamp circuit was kept 5 per cent. faster than the frequency with which the condenser was mechanically discharged. Fig. 1 shows the response of the sphincter to pairs of stimuli. Five minutes' rest was allowed between each response. Fig. 1 A shows the effect of shortening the interval between the pair. Fig. 1 B shows the effect of increasing intensity of stimuli on the pedal edge, and Fig. 1 C the effect of intensity for stimuli applied over the sphincter itself.

When stimuli are applied at some distance from the sphincter, increase in intensity has no effect upon the size of the contraction, as already shown (Pantin, 1935a) (Fig. 1 B). But if the intensity is raised to about ten times the threshold value, the primary response may be followed by one or more extra contractions. A very strong stimulus may thus give rise to a brief train of two or three impulses usually about $\frac{1}{2}$ sec. apart, instead of to a single impulse (Fig. 1 D). Whether this after-discharge has the same origin as that discussed in an earlier paper (Pantin, 1935b) is uncertain.
For stimuli of threshold value the response of the sphincter is the same whether stimuli are applied over the muscle itself or at some distance from it. There is no response to the first stimulus, and the response to the second depends upon the time interval. But for strong stimuli directly over the muscle the response is no longer independent of the intensity as it is for indirect stimulation (Fig. 1 C). At about two to four times the threshold intensity, a distinct local contraction of the sphincter muscle becomes evident at the first stimulus. At the same time, the response to a second or subsequent stimulus increases above that obtained at the threshold. This is partly or entirely due to local contraction of the sphincter muscle in the neighbourhood of the electrodes, as in the response to the first stimulus. With still stronger stimuli these effects become greater till at five to ten times the threshold value quite strong contractions can be recorded in response to a single stimulus (Fig. 1 E). At these high intensities there is again a tendency for repetitive excitation.

A single strong stimulus when applied over the sphincter causes that muscle to respond. Since such a strong stimulus may sometimes give rise to repetitive excitation it might be thought that this was always the cause of any response which took place. For if a single stimulus initiated two or more impulses instead of one, the muscle would certainly contract. A single strong stimulus might in this way produce a response, where a weaker one, which could only initiate a single impulse, would produce none. This explanation is not adequate. The response to a single strong stimulus immediately follows the stimulus itself. If, therefore, two or more impulses had been set up these must follow each other very rapidly, but such a rapid sequence of impulses would produce much greater contraction than that which is actually observed (cf. Fig. 1 A) nor would the contraction be a local one. The response to a single stimulus in this case is therefore not due to a series of impulses set up in the nerve net, and since such a stimulus can only cause a contraction of the muscle when it is applied directly over it, it seems that the contraction must be the effect of direct excitation of the muscle fibres.

A single stimulus can thus excite the muscle if applied directly to it. The reason a single impulse fails to cause a response when it reaches the muscle indirectly from the nerve net is not therefore due to the state of the muscle being one in which contraction cannot take place. Since in this case the excitation wave certainly gets as far as the muscle itself we must suppose that there is a failure of conduction at the neuromuscular junction. The response to a second impulse must therefore be due to the facilitating effect of the first; though the latter is unable to pass the neuromuscular junction it must leave behind it an effect which admits the passage of the following impulse to the muscle fibres. The height of contraction is in fact a statistical measure of the success of this second impulse.

Neuromuscular facilitation alone accounts for the first step of the staircase. Whether the size of the further contraction steps following succeeding stimuli is due solely to neuromuscular facilitation or whether it is an addition affected by previous contractions, as in staircase of the heart, cannot be definitely settled. But the size and time relation of each step in a series of contractions are so similar to the first step that it seems unlikely that such a new factor enters in to any great extent.
DISCUSSION.

We may now consider more closely the relation of the response of the sphincter of Calliactis to the contractions of the bell of the Medusae. Romanes (1877) showed that the Medusa of Aurelia from which otocysts had been removed gave a well developed “staircase” in response to stimuli of threshold value (Fig. 2). He specifically set before himself the question as to whether the staircase effect was due “to the occurrence of previous stimulations or to that of previous contractions”. He showed that the staircase was not influenced by throwing in the successive stimuli at different places on the bell of the medusa, and proved by this “that the effect was a general one pervading the whole mass of the contractile tissue and not confined to the locality which is the immediate seat of stimulation”. On the other hand, he drew attention to the fact that the first steps in the staircase involved no contraction of the muscle and argued from this that the staircase depended on the “process of stimulation as distinguished from that of contraction”.

Fig. 2. a, staircase in Medusa of Aurelia (marginal bodies removed). Contractions recorded on stationary drum. From Romanes: stimulation signals inserted from Romanes' text description. Stimulation interval 3 sec.: first two stimuli ineffective. Note lack of fusion of twitches. b, staircase of sphincter of Calliactis. Stimulation interval 1.2 sec. Note partial fusion of twitches. c, summation in the slow longitudinal mesenteric muscles of Calliactis. Stimulation interval 1.8 sec. Note complete fusion of individual twitches.

In these things the Medusa bell resembles the sphincter of Calliactis, and our conclusions concerning the latter are implicit in Romanes' conclusions. Further, Romanes' experiments show that by increasing the intensity of the stimulating current a response is obtained to the first stimulus of a series. In Aurelia, muscle as well as nerve net lie beneath the stimulating electrodes, and it is probable that we have here evidence of response of the muscle to direct stimulation, as in the sphincter of Calliactis.

The relation of the muscular response of the bell of Aurelia to that of the sphincter of Calliactis is shown in Fig. 2. In the Medusa, the staircase in response to a series of shocks consists of discrete contractions, each of which is greater than the last. In the sphincter of Calliactis, on the other hand, the duration of the whole “twitch”, including relaxation, is so long that these fuse partially to give a step-like increase in tension. The condition which governs the relation of these two types of staircase is the relative duration of the time for a complete muscular twitch to the time interval between shocks which is necessary to cause a facilitated response of the muscle. In the Medusa bell the time for a complete twitch is short compared
with the stimulation interval which will still produce a facilitated response. In the sphincter of *Calliactis*, on the other hand, the stimulation interval required for a facilitated response is greater than the time required for contraction, though less than the time required for the whole twitch including relaxation.

Taking the sphincter of *Calliactis* as a central type, we can arrive at the condition in the bell of *Aurelia* by increasing the stimulation interval at which facilitation can take place till the muscular twitches to successive shocks no longer fuse. The experiments of Bethe (1935) suggest that the condition in the Medusa *Cotylorhiza* is similar to that in *Aurelia*, but that there is a further differentiation of the radial and circular muscles in the bell of this Medusa. Both these muscles take part in the normal contraction. The staircase of the circular muscle appears to resemble that described by Romanes in *Aurelia*. The first stimulus may produce no response. The staircase of the radial muscles, on the other hand, seems to be much less marked. Even the first stimulus of a series produces a contraction of considerable extent. We might express this by saying that the interval at which a response can still be facilitated has become very long, so that after even a long period of rest the first shock is able to activate a large proportion of the muscle fibres. If this process were extended till the stimulation interval at which facilitation took place were infinitely long, every stimulus would provoke a complete contraction of the whole muscle, and we would thus arrive at a condition exactly parallel to a single motor unit of vertebrate skeletal muscle. By proceeding from the sphincter of *Calliactis* in the opposite direction from this we can arrive at a condition in which the duration of a muscular “twitch” is very long compared with the interval required for facilitation. Under these conditions steps such as those found in the sphincter contraction fuse completely, and we get a smooth sigmoid curve. Such a response is in fact found in the mesenteric muscles of *Calliactis* (Fig. 2).

We have chosen these examples from among the Coelenterata, but responses of a similar kind can be found in the muscles of other groups. Except for the fact that the phenomena are observed to take place at much lower frequency, there is no essential difference between the case of the mesenteric muscles of *Calliactis* and that of crustacean muscle (Lucas, 1917), and even vertebrate skeletal muscle passes into a similar condition under the influence of gross fatigue or abnormal conditions such as partial curarisation (Adrian and Lucas, 1912). In all these cases there is strong evidence for the summation of the effect of propagated disturbances at the neuromuscular junction. The staircase phenomena in Coelenterata seem to present a variety of conditions which owe their origin to neuromuscular facilitation and the analogy with the staircase in the vertebrate heart is superficial.

**SUMMARY.**

1. The relation of the “staircase” effects in the vertebrate heart, in the bell of Medusae and in the sphincter of *Calliactis* is discussed. Whereas in the heart the effect is due to an intrinsic increase of contractility of the whole contractile tissue, evidence is brought to show that the staircase in *Calliactis* and in Medusae depends
upon neuromuscular facilitation by which each stimulus in a series reaches more and more individual muscle fibres.

2. The relation of different types of contraction in coelenterate muscle is discussed. It is shown how this depends upon the relation of the stimulation interval necessary for a facilitated response and the duration of a complete contraction and relaxation of the muscle. If these are about equal the step-like contraction of the sphincter of *Calliactis* results; if the facilitation interval is long a staircase similar to that of a Medusa bell is produced; if the duration of the twitch is relatively long a smooth contraction as in the mesenteric muscle of *Calliactis* is produced.

REFERENCES.

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