THE NERVE NET OF THE ACTINOZOA

V. TEMPERATURE AND FACILITATION IN METRIDIUM SENILE

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(With Three Text-figures)

INTRODUCTION

In the anemone Calliactis parasitica stimulation may lead to a series of protective responses. These responses are controlled by neuromuscular facilitation between the nerve net and the muscles (Pantin, 1935). In the paper where this was shown it was pointed out that the protective responses of Metridium as described by Jordan (1908) and Parker (1918) might very well fit into the same scheme, except that the relative importance of the muscles was different from that in Calliactis. In the present paper it will be shown that the responses of Metridium senile obey the same general rules which were found to be true in Calliactis. Indeed, certain features are shown with even greater clearness. For this reason, the responses of the longitudinal muscles of Metridium were further chosen for an investigation of the influence of temperature on neuromuscular facilitation. Experiments on Calliactis suggested that neuromuscular facilitation possessed a large temperature coefficient. Apart from the intrinsic interest of the effect of temperature on this process, a systematic investigation of this factor is of importance, since any change in facilitation influences the whole behaviour of the animal. It will be shown that this influence is considerable.

THE RESPONSES OF METRIDIUM SENILE

Whereas in Calliactis the primary protective response is a rapid and powerful closure of the marginal sphincter, in Metridium protection is brought about by an even more rapid and powerful contraction of the longitudinal muscles of the mesenteries. The sphincter in Metridium is a very slow muscle and only completes the closure of the disc after this has been already invaginated by the contraction of the mesenterics. Parker describes retraction in Metridium as being initially due to the action of the mesenteric muscles which depress the oral disc, followed by the parietals which act on the column wall. After depression of the disc has proceeded somewhat, the contraction of the sphincter over the invaginated disc completes the process. The results of mechanical stimulation on M. senile agree closely with Parker's
results on *M. marginatum*. It is possible however to obtain by mechanical stimulation a considerable variety of response. Sudden mechanical stimulation of the pedal edge, if not too extensive, may cause one or two contractions of the longitudinal mesenteries without any obvious response of the other muscles. After the mesenteries have ceased to respond, the animal returns to its original form. Greater and more prolonged stimulation calls up the response of all the muscles in the way Parker describes. In addition to this, it is also possible to cause a contraction of the parietal muscles by themselves. Prolonged gentle stroking can cause a gradual shortening of the column of the anemone without invagination of the disc. The sphincter, on the other hand, never seems to contract without the longitudinal mesenteries.

Though the muscles are morphologically very different in situation, the contraction of the longitudinal mesenteries of *Metridium* closely resembles the contraction of the sphincter of *Calliactis*. The fact that the response does not take place continuously but as a series of discrete sudden contractions ranging round 1–4 per second is even more obvious in *Metridium* than in *Calliactis*. As in the latter, this indicates the arrival of successive nervous impulses at the muscle.

**ELECTRICAL STIMULATION**

Stimuli were usually given by a metronome-controlled discharge in a neon-lamp circuit described by Pantin (1935). Later a more convenient method was found to be simple condenser discharges controlled by a special relay. This was a combined relay and signal marker recording on the smoked drum alongside the record of the contractions. Fig. 1 shows the signal relay and its connexions. It is actuated either by a metronome or a commutator. When in the resting position it permits a $1\mu$ F. condenser to be charged. When the armature is attracted, this contact is broken and the condenser is allowed to discharge through a resistance of 5000 ohms in series with a potentiometer of 400 ohms. The advantage of this system is that the precise moment of stimulation is directly recorded mechanically on the smoked drum.

All responses were recorded by a weak "isometric" lever. This method of recording has the advantage that the muscle is brought back to the same length after each contraction. But the records are not simple records of the isometric tension developed because the weakness of the lever permits some movement. Thus in Fig. 2 the length of the mesentric muscles between the disc and the base of the animal was 5 cm. The maximum contraction shown in the figure corresponded to a shortening of 0.5 cm. Such maximal contractions are very powerful. With a rigidly isometric lever which does not permit shortening, the recording thread may tear itself from the disc of the anemone.

In their behaviour to electrical stimulation the longitudinal mesenteries of *Metridium* resemble the sphincter of *Calliactis* in great detail. In both a single effective stimulus produces no response in the muscle, but in both a second stimulus causes a response if it follows the first at a suitable interval. The size of the response decreases from an optimum as the interval between the stimuli is lengthened (Figs. 2 and 3). A succession of stimuli in both cases causes increasing contractions of the muscle through facilitation. Owing to the rapidity and great size of the contractions
of the longitudinal mesenteries in *Metridium* the effects are indeed much more evident than in the sphincter of *Calliactis*.

Electrical stimulation calls up graded responses of the various muscles as in *Calliactis*. When the column is stimulated, the threshold for the response of each muscle appears to be the same. Whether a rapid contraction of the longitudinal mesenteries is called up, or a symmetrical shortening of the column of the anemone owing to the contraction of the parietals, or the slow contraction of the sphincter, the intensity of the stimulus is without effect. According to Jordan (1908) the mesenteric and parietal muscles are successively brought into action with increasing intensity of stimulation; the mesenterics having a lower threshold. In the present

![Diagram](image)

Fig. 1. Combined recording signal and relay for condenser discharges. The relay magnets are in circuit with a commutator or metronome, C. At rest, the armature A permits condenser to charge. When the armature is attracted this circuit is broken and the condenser discharges via mercury contact B. The discharge takes place through a 5000Ω resistance and a potentiometer D (400Ω). From D arise the stimulating electrodes (Ag/AgCl). The armature carries a light arm writing on the smoked drum. E, anemone in sea water attached to lever.

experiments this was never found. The relative response of the muscles depends not on thresholds but on facilitation. As in *Calliactis*, the controlling factors are the number and frequency of stimuli applied to the nerve net. The main difference between *Metridium* and *Calliactis* is due to the fact that the most rapid reaction of the former concerns the longitudinal mesenteries and not the sphincter. At 15°C the longitudinal mesenteries begin to facilitate at about 1 stimulus in 3 sec. in *Metridium*, the size of each contraction increasing as the frequency rises above this. The parietal musculature, on the other hand, comes into action at frequencies well below this, being perceptible at 1 stimulus in 10 sec. These muscles cause shortening of the column, the disc remaining expanded (Fig. 2).

In contrast with *Calliactis*, the sphincter in *Metridium* is an exceedingly slow muscle. Its contraction phase occupies one or two minutes, and its relaxation time
may be far longer than this, even when the muscle is subjected to a considerable force tending to relax it. Nevertheless, the sphincter appears to become facilitated by a range of frequencies which corresponds roughly to that required to activate the rapidly acting longitudinal mesenteric muscles. It readily responds to rates of stimulation between 1 in 3 and 1 in 1 sec. Owing to the slowness of action of the muscle, the contractions take place long after the stimuli are given. Consequently the sphincter contraction does not appear till after the mesenteries have already pulled in the disc, so that the fact that the sphincter completes the retraction process is primarily a mechanical consequence of the relative speeds of action of the two muscles.

Fig. 2. A. "Staircase" of longitudinal mesenteries: four stimuli 20 sec. interval between each.
B. Parietal contraction: eight stimuli 40 sec. interval.
C. Five stimuli at 10 sec. interval. Very slow drum. Recording thread at right angles to column to record sphincter. L, longitudinal mesenteric response partially recorded; S, subsequent sphincter response to same stimulus.
D. Response of longitudinal mesenteries to 2 stimuli at 24° C. Stimuli 0.6 sec. apart.
E. The same at 5° C. Stimuli 3 sec. apart. Note slow contraction and relaxation.

The conditions for activation of the longitudinal mesenteric and the sphincter, however, are not altogether the same. The response of the former to a series of stimuli is invariable, whereas the sphincter contractions are apt to vary considerably to successive stimuli of the same frequency. This is at least partly due to the effects of previous stimulation. The response of the sphincter to a given series of stimuli is generally less in the resting anemone than in one that has been repeatedly stimulated. The cause of this variability is difficult to ascertain, though it may be connected with some degree of interneural facilitation between the column nerve net and the sphincter. But it is very difficult to record successive responses of the sphincter under even approximately the same mechanical conditions owing to the extensive
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deployment of the animal by the responses of the longitudinal mesenteries to the same stimuli.

Apart from these complications, the range of frequencies required to facilitate the sphincter is the same as that required for the longitudinal mesenteries. Frequencies which are too low to bring in the longitudinal mesenteries are also too low to bring in the sphincter. Thus in one experiment a succession of 50 shocks at a frequency of 1 in 4 secs. brought about a complete contraction of the parietal musculature in a fully expanded *Metridium*. Neither the longitudinal mesenteries nor the sphincter showed any sign of response. At the end of this sequence of stimuli, the frequency was abruptly raised to 1 in 1 sec. for 3 or 4 stimuli. The

![Fig. 3](image.png)

*Fig. 3.* Responses of longitudinal mesenteries to pairs of stimuli at different intervals and at different temperatures. Note profound effect of temperature on duration of facilitation.

longitudinal musculature responded by its usual powerful contractions, and this in turn was followed by the sphincter.

As in the sphincter, the contraction of the circular muscle of the column takes place extremely slowly. It can be brought into action by very low frequencies, as in *Calliactis*, but the response is extremely variable and has not yet been fully analysed.

At high frequencies (1 in 1 sec.) corresponding to the range of the longitudinal mesenteries, not only is the sphincter brought into action, but also to some extent the longitudinal muscles of the tentacles and the radial musculature of the disc. In *Calliactis* it has been shown that the nervous impulse from a stimulus applied to the column may sometimes get to the longitudinal muscles of the tentacles. But the effect is very much more striking in *Metridium*, for almost all the tentacles may be affected; and a few stimuli may cause the disc and tentacles to shrivel into an
opaque flat mass. In the white variety of this anemone, the transition from the transparent expanded tentacles to a white opaque state is very remarkable.

These observations show that the protective responses in Metridium indicate the presence of essentially the same mechanism as in Calliactis. The succession of the responses in the protection of the disc is primarily determined not by the threshold but by the number and frequency of stimuli.

As in Calliactis, these responses of the column are all symmetrical about the axis of the animal, so far as they have been observed here. The responses of the column in Metridium indicate the presence of a through conduction system such as is found in Calliactis. As in Calliactis also, this system does not extend over the disc, with the exception that the longitudinal musculature of the tentacles is accessible to stimulation of the column. On the other hand, the feeding and other responses of the disc all show marked localization of response. There is no evidence of extensive through conduction in this region. As in Calliactis, a group of impulses set up by stimulation at any point on the disc appears to become gradually diminished in number when conducted to adjacent regions of the disc, till at sites far removed from the stimulus the whole group becomes extinguished.

THE EFFECT OF TEMPERATURE

The effect of temperature was studied on the longitudinal mesenteric muscles of Metridium. Before an experiment, the anemones were placed on glass plates to which they adhered, and a recording thread was sewn through the edge of the oesophagus. They were placed in a cylindrical vessel containing about 2 litres of sea water, which was in turn immersed in a large cylindrical vessel of capacity about 15 litres. This outer bath was maintained at any temperature required.

The animals were left for 20-30 mins. in sea water at the desired temperature before any experiment was commenced. A thermometer inserted through a hole cut in the side of the body wall of a control animal showed that this allowed ample time for the internal temperature of the anemone to reach equilibrium with the surrounding sea water. An anemone was then attached to the lever and allowed to expand. During the course of an experiment at any one temperature the water round the anemone was continually stirred and its temperature maintained within a range of half a degree. The range of temperature investigated was from 5 to 25° C. Below this it is difficult to obtain any response from the animal, while death occurs between 25 and 30° C.

Temperature has a profound effect upon both contraction and facilitation. Fig. 2 records two contractions, one at 24° C. and the other at 5° C. At the low temperature, contraction and relaxation are both much slower. Relaxation is always a much slower process than contraction so that the effect of temperature on relaxation is the most evident. The temperature coefficient of both processes is, however, about $Q_{10} = 2.0$. The factors governing the velocity of contraction and relaxation are complex and there is no object at present in analysing this temperature coefficient further.
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The effect of temperature on facilitation is very striking, both in its magnitude and in its consequences. Fig. 3 shows a series of responses to pairs of stimuli at different time intervals at each of the three temperatures 5, 15 and 24° C. It will be observed that though temperature affects the facilitation enormously, the rule that a single stimulus is ineffective is always maintained. But at low temperatures the facilitating power of a single stimulus endures for an extraordinarily long time, so that a second stimulus may cause a response of the longitudinal mesenterial muscles even 10 sec. after the first.

As the longitudinal mesenteries require a higher frequency than any other muscles in the animal to facilitate them, it is evident that at low temperatures an extraordinarily low frequency of stimulation may be sufficient to keep the entire musculature of the animal in prolonged tonic contraction. The slow relaxation rate at low temperatures aids this.

At all temperatures a stimulus succeeds in producing a response if it has been preceded by a previous stimulus by not too great an interval of time. In general the longer the interval between the stimuli, the smaller is the response to the second stimulus likely to be. But in comparing the response of the longitudinal mesenteries at high and low temperatures, an important difference is to be seen. At 20–25° C. the height of the contraction following a pair of stimuli increases as the stimuli follow one another more closely almost up to the absolute refractory period. The sphincter of *Calliactis* behaves in the same way. Fig. 3 shows, however, that there is a significant change from this relation at low temperatures. The size of the contraction increases progressively as the stimulation interval is shortened up to a certain point. This point, however, is reached long before the absolute refractory period, and a further shortening of the stimulation interval produces no serious change in the size of a contraction. This "plateau" is clearly seen in Fig. 3 at 5 and at 15° C. In *Calliactis* this "plateau" effect is much less evident, and the size of a contraction progressively falls from an optimum as the time interval between the stimuli increases.

Table I

<table>
<thead>
<tr>
<th>Temp. °C.</th>
<th>5°</th>
<th>7°</th>
<th>10°</th>
<th>17°</th>
<th>21°</th>
<th>24°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulation interval for response of 5% maximal tension, in sec.</td>
<td>8.0</td>
<td>6.0</td>
<td>4.6</td>
<td>2.0</td>
<td>1.2</td>
<td>0.9</td>
</tr>
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It is a matter of some interest to attempt to find a temperature coefficient for the rate of decay of the facilitation process. Until more definite knowledge of how the process takes place has been obtained, any method of measuring the degree of facilitation is to some extent arbitrary. For convenience, at each temperature, the interval between a pair of stimuli was found which was just long enough for the facilitated response to the second stimulus to have fallen to a small but definite fraction of the maximal contraction. This gives a measure of the rate of decay of facilitation. Table I gives the stimulation intervals at which 5 per cent maximal tension is achieved at different temperatures in a typical experiment. It shows that
the process has a high temperature coefficient of $Q_{10} = 3 - 3.2$. If such results as these are taken and the logarithms of the intervals plotted against the reciprocal of the absolute temperature, values for a "critical thermal increment" can be calculated (Crozier, 1924). The data plotted in this way tend to fall fairly regularly on a straight line and indicate an increment of about $\mu = 18,000$. But the processes which determine the rate of decay of facilitation have not yet been ascertained, so it is not certain to what process, if any, such a critical thermal increment can be attached. Thus the decay interval, as measured here, might be related to its underlying processes in a complex way, or the interval might be dependent upon the sum of the times occupied by more than one event in temporal succession. Should either of these conditions be found true, a numerical value of $\mu$ will have no special significance.

**SUMMARY**

1. An account is given of the responses of *Metridium senile* to mechanical stimulation. The responses resemble those described by Parker in *M. marginatum*. The chief protective response is due to contraction of the longitudinal mesenteric muscles and not to contraction of the sphincter as in *Calliactis*.

2. A simple apparatus for stimulation of Actinozoa is described.

3. The behaviour of *Metridium* to electrical stimulation resembles that of *Calliactis*. The response does not depend upon the intensity of individual shocks but on their number and frequency. As with *Calliactis*, *Metridium* obeys the rule that a single stimulus produces no effect. The longitudinal mesenterics of *Metridium* resemble the sphincter of *Calliactis* remarkably closely in their properties.

4. As in *Calliactis*, graded responses can be produced in *Metridium* by varying the frequency of stimulation. The longitudinal mesenteries require the highest frequency for a facilitated response. The parietals require a lower frequency. The sphincter requires a relatively high frequency of stimulation but its action is delayed owing to its slow rate of contraction. The graded responses of different muscles to stimulation are controlled by facilitation. They are not connected with differences in threshold.

5. Temperature has a profound effect on the responses of actinozoans. Both contraction and relaxation are slowed at low temperatures ($Q_{10} = 2$). The facilitating effect of a stimulus endures more than three times longer for a fall in temperature of $10^\circ$ C. Temperature thus greatly influences the rate of decay of the facilitation process.

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**REFERENCES**


