

STUDIES ON THE MYONEURAL PHYSIOLOGY OF ECHINODERMATA

II. CIRCUMORAL CONDUCTION IN *CUCUMARIA*

BY WALTER POPLÉ AND D. W. EWER*

Department of Zoology, University of Natal, Pietermaritzburg

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The properties of a neuromuscular preparation of the mid-ventral pharyngeal retractor muscle of *Cucumaria sykion* (Lampert) have been previously described (Pople & Ewer, 1954). When excited by stimulation of the radial nerve, this muscle shows a double response consisting of a quick component, tentatively attributed to direct nervous paths through the motor complex of the muscle and a delayed component which possibly arises from secondary paths within the motor complex itself. This characteristic double response can be clearly seen in the contractions recorded in the upper trace of Fig. 2 of the present paper. The tension developed by this muscle is dependent upon the intensity of stimulation applied to the radial nerve. No evidence of frequency sensitive facilitation at the myo-neural junction was found.

The present paper is concerned with a study of the responses of the retractor muscles to impulses conducted around the circumoral nerve ring. For this purpose two types of preparation have been used.

Double-muscle preparation. This consists of the mid-ventral and left ventral retractor muscles and their corresponding radial nerves together with the section of the circumoral nerve between the two muscles. The method of preparation is similar to that previously described for the single muscle preparation. To prevent rupture of the circumoral nerve it has been found best to hold the preparation down with a piece of plastic sheeting stretched over the ossicles and the sector of the pharyngeal mass.

Five-muscle preparation. This consists of all five retractor muscles. Five cuts are made from the cloaca anteriorly through the inter-radial integument to the lip of the oral cavity. The viscera and the longitudinal strips of integument posterior to the retractor muscles are cut away, leaving the pharyngeal mass and its five retractor muscles. The five radial nerves which lie beneath the longitudinal radial muscles are dissected out as in other preparations. The preparation is impaled upon a glass spike passing through the oral lip, oral cavity and oesophagus and is prevented from twisting by a star-shaped piece of plastic sheeting, the points of which pass between the muscles and are held down by pins.

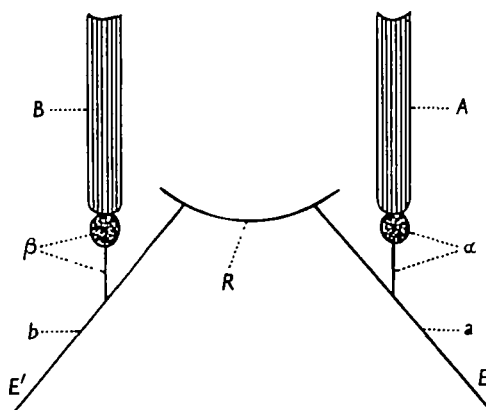
Light auxotonic levers were used throughout. The tension developed by a muscle depends upon the initial stretch. In most preparations the levers were adjusted so that all muscles were stretched to the same initial length; this was not however always done (e.g. Fig. 4). The lever system was usually adjusted so that a shortening

* Present address: Rhodes University, Grahamstown, South Africa.

of 1 mm. developed a tension of 1 g. Fine platinum wire electrodes were used. The cathode was placed beneath the radial nerve and the anode upon the longitudinal radial muscle immediately above the cathode. The electrodes were normally placed 0.2–0.5 cm. from the motor complex. All experiments were carried out at temperatures of 20–23° C.

THE CHARACTERISTICS OF CIRCUMORAL CONDUCTION

The general anatomical organization of a double muscle preparation is shown in Fig. 1. If a suitable stimulus is applied to the radial nerve *a* at *E* both muscles *A* and *B* will contract. Nerve impulses to the ipse-radial muscle *A* will pass along the radial nerve, the motor nerve and through the motor complex α . Impulses to the para-radial muscle *B* will pass along the radial nerve to its point of union with the circumoral nerve *R*, along this to the next radial nerve and then along the radial nerve *b* to the motor nerve and motor complex β . A comparison of the responses of muscles *A* and *B* will therefore give information about the characteristics of circumoral conduction. Histologically the circumoral and radial nerves are composed of the same neural elements, as Smith (1937) has shown in *Marthasterias glacialis* (L.).



Effect of intensity. If, with such a preparation, a single shock well above threshold is applied to the radial nerve *a*, both ipse-radial and para-radial muscles will contract. Both responses show the quick and delayed components previously described in the account of a single muscle preparation. If the stimulus intensity is lowered until it is just above threshold the ipse-radial muscle will respond with a small contraction, while the para-radial muscle may show a minute response. With increasing intensities of stimulation the tensions developed by both muscles increase until a maximum is reached. The same result is obtained with batteries of stimuli (Fig. 2). It will be noted that the maximal tension developed by the para-radial muscle is never as great as that which this muscle can develop with supra-maximal stimulation

of the radial nerve of its own radius. Further, it may be seen (Fig. 3) that although the tension developed by the para-radial muscle is less than that developed by the ipse-radial muscle, both achieve maximal tension at about the same intensity of stimulation of the radial nerve.

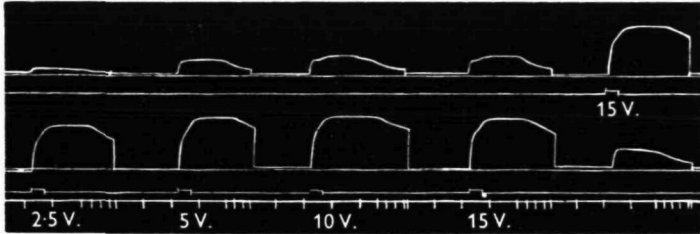


Fig. 2. *Cucumaria* pharyngeal retractor muscles. Responses of two adjoining pharyngeal retractor muscles recorded one above the other. Volleys of stimuli of various intensities are applied to one radial nerve. In the first four responses the radial nerve supplying the muscle recording the lower trace was stimulated; in the last response the stimulus was applied to the radial nerve supplying the muscle recording on the upper trace. Stimulation frequency 2.5 s./sec. Ten stimuli in each battery of shocks. Time marker 10 sec.

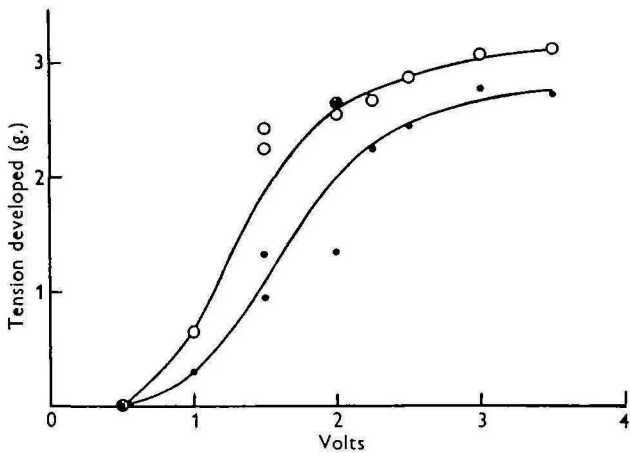


Fig. 3. Graph showing the relation between stimulus intensity and tension developed in an ipse-radial muscle (open circles) and the adjoining para-radial muscle (full circles). When stimulated by way of its proper radial nerve the para-radial muscle developed a maximal tension of 4.75 g. Stimulation with 10 shocks of 2 msec. duration at a frequency of 2.5 s./sec.

Effect of frequency. If the frequency instead of the intensity of stimulation is altered, it is found that the responses of the para-radial muscle change in a similar manner to those of the ipse-radial muscle. This effect is shown in Fig. 4. At very low frequencies of stimulation both muscles respond with incompletely fused contractions; at 1 stimulus/sec (s./sec.) the response is almost smooth and smooth contractions, neglecting the humping caused by the double character of the response, are obtained at 2.5, 5 and 10 s./sec. At 50 s./sec. the tension developed by both muscles is less than at 5 s./sec. This depression of the responses at high frequencies of stimulation has already been described in the single-muscle preparation.

Conduction velocity. It is possible to make an approximate assessment of the conduction velocity of the impulses releasing the quick response in their passage along the circumoral nerve. It is, however, necessary first to show that the inter-radial paths do not pass by way of the motor complex. Thus in Fig. 1 impulses originating at *E* might pass directly along the radial nerve to the circumoral nerve or relay

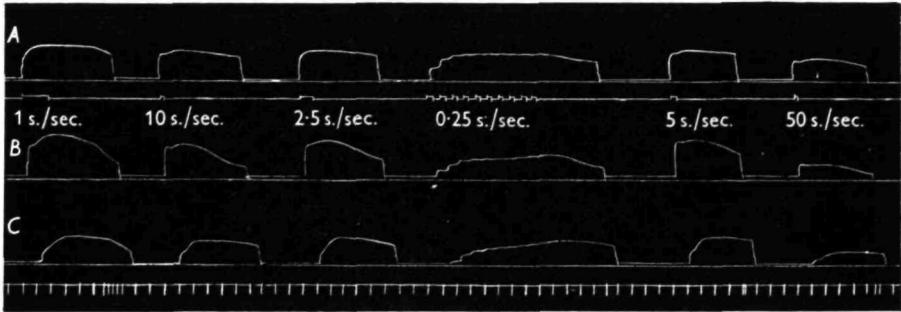


Fig. 4. Extract from a five-muscle preparation. The radial nerve of the mid-ventral muscle, recorded on the upper trace, was stimulated with batteries of 10 stimuli of 2 msec. duration at different frequencies. The two lower traces record the responses of the right ventral (*B*) and right dorsal (*C*) muscles. The muscles have been unequally stretched so that the lever excursions recorded by each are approximately equal. Stimulus intensity 8 V. Time marker 5 sec.

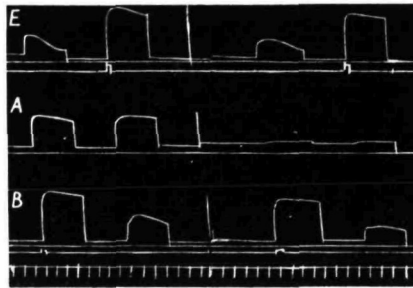


Fig. 5. Records from the mid-ventral (*A*), left ventral (*E*) and right ventral (*B*) retractor muscles. In the first two responses the radial nerve to *B* and then to *E* is stimulated as shown by the relevant signal markers. The motor nerves and motor complex of *A* were then almost completely destroyed by cautery and the radial nerves of *B* and *E* again stimulated. Stimulus in each case a battery of 10 shocks of 2 msec. duration at 15 V. Time marker 10 sec.

through the motor complex α . Similarly, impulses passing from one radius to a more distant one might relay through each succeeding motor complex in turn. That this does not occur is demonstrated in Fig. 5. In a preparation of the mid-ventral, right and left ventral muscle two test shocks were applied; first to the left and then to the right ventral radial nerve. The motor nerve and complex of the mid-ventral muscle were then destroyed almost completely by cautery. It can be seen that the cauterization does not markedly affect the transmission between the right and left ventral radii. Similarly, it may be shown, in a double-muscle preparation, that destruction by cautery of one motor complex does not affect the responses from the para-radial muscle to stimulation of the radial nerve whose motor complex has been

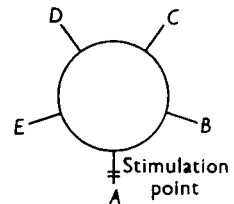
destroyed. It may be taken then that the path of excitation from one radius to another more distant passes only by way of the radial and circumoral nerves without relay through the motor complex.

The velocity of conduction along the circumoral nerve may be determined in a three-muscle preparation. Calling the three radii *A*, *B* and *C*, a stimulus is applied to radial nerve *A* and the difference in latent period of response between muscles *B* and *C* is determined. This time difference will be the time taken for conduction around one-fifth of the circumoral nerve ring. Measurements made on seven preparations gave a mean value of 0.11 ± 0.04 m./sec. at $20-23^\circ$ C. It must be emphasized that this value is very approximate as considerable uncertainty attaches to determinations of the length of the nervous tracts owing to the contractility of the tissues around them. This value may be compared with that of 0.17 ± 0.03 m./sec. previously determined for the conduction velocity along the radial nerve of impulses releasing the quick response. It is clear that the velocities are of the same order.

As has been previously emphasized, determination by mechanical methods of the conduction velocity of the impulses releasing the delayed response is difficult. Two determinations made previously suggested that there was no significant difference between the conduction velocities along the radial nerve of the impulses releasing the two types of response. Evidence that these conduction velocities are also of the same order along the circumoral nerve ring is obtainable with a five-muscle preparation. If a strong stimulus or a battery of stimuli be applied to one radial nerve, all five muscles will contract. The time between the onset of the quick and delayed responses is found to be almost the same in all five muscles (Table 1). Certainly there is no evidence that the interval is greatest in the muscles most distant from the stimulated radius.

Table 1. *Time delay between the onset of the quick and delayed responses of the muscles of a five-muscle preparation when a supra-maximal stimulus is applied to the radial nerve of one muscle*

Muscle	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>
Delay (sec.)	2.1 ± 0.3	2.9 ± 0.3	2.1 ± 0.3	2.0 ± 0.6	2.6 ± 0.3



Delayed facilitation. In the single-muscle preparation a delayed facilitation of the slow response was described. If two single stimuli are applied at intervals up to about 60 sec. the magnitude of the delayed response to the second stimulus is greater than that to the first.* Evidence has been presented which suggests that the site of this facilitatory effect lies in the motor complex. The phenomenon may be analogized with post-tetanic potentiation. A similar facilitation of the delayed

* This effect is not found for all preparations and may appear in only one or two muscles of a five-muscle preparation. The cause of this variability is being investigated.

response of the para-radial muscle in a double-muscle preparation may also be demonstrated. Moreover, it is possible to facilitate the response given by a muscle to stimulation of its own radial nerve by previous stimulation of the para-radial nerve and vice versa.

From these experiments it appears that conduction around the circumoral ring is very similar to that along a radial nerve. In both cases a muscle shows quick and delayed responses; in both tension recruitment is dependent upon the intensity and not the frequency of stimulation; in both delayed facilitation is found and the conduction velocities of impulses are of the same order. These findings are not unexpected as the radial and circumoral nerves are histologically similar. One important point, however, does emerge, namely, that in these conditions stimulation never releases maximal possible tension from a para-radial muscle. In other words conduction around the circumoral ring appears to be 'decremental'.

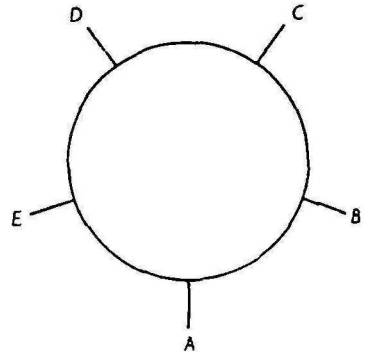
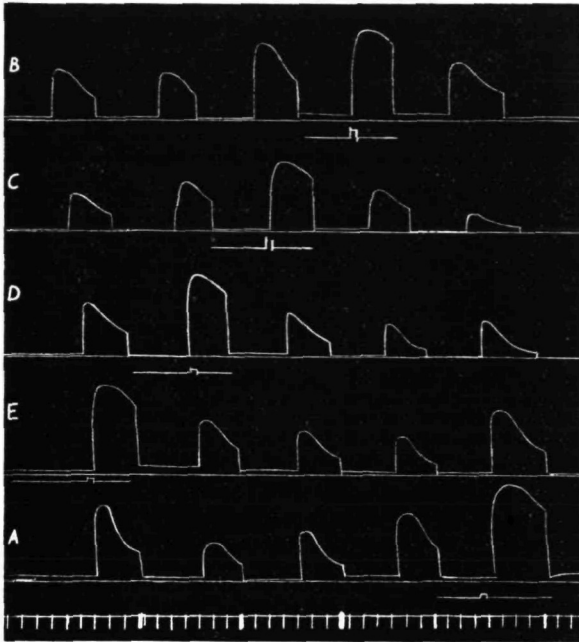
DECREMENTAL CONDUCTION

If in a five-muscle preparation the mid-ventral radial nerve is stimulated at an intensity above threshold all five muscles contract. The tension developed by the right and left ventral muscles is less than that developed by the mid-ventral muscle, while that developed by the right and left dorsal muscles is smaller still. That this effect is symmetrical around the ring can be seen in Fig. 6*a* where a supra-maximal shock was applied to each radius in turn.

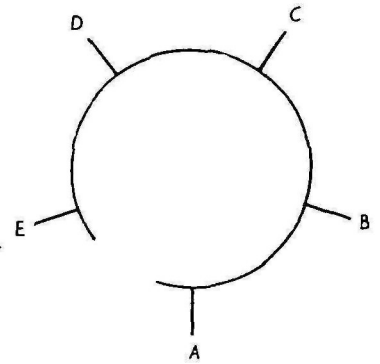
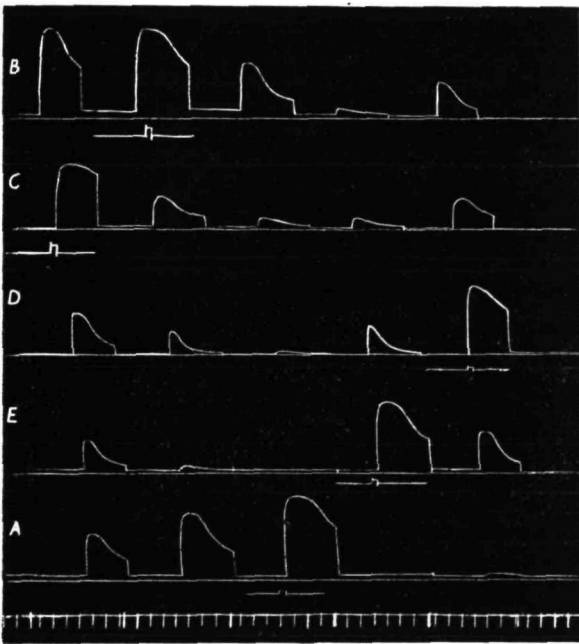
Fig. 6*b* shows responses from the same preparation after cutting the circumoral ring between radii *A* and *E*. A complete series of decremental stages around the ring may be seen in the responses obtained on stimulating radial nerves *A* and *E*. In trace 6*b* a minute response is given by muscle *A* when the radial nerve supplying muscle *E* is stimulated. No corresponding response from muscle *E* after stimulation of radial nerve *A* was recorded in this case, but the examination of results from further preparations shows that complete transmission does frequently occur. Moreover, it will be seen that the response obtained from stimulating nerve *C*, which lies immediately opposite the cut, is almost unchanged.

As with the double-muscle preparation, all five muscles respond when a single shock is applied to one radial nerve and the tensions developed in each of the five muscles depend upon the intensity and not the frequency of stimulation applied to any one radial nerve.

The simplest interpretation of these various results which can be offered is that the decremental effect depends simply upon the geometrical arrangement of the fibre tracts running from a radial nerve to its own and other motor complexes. This is illustrated in diagrammatic form in Fig. 7. From radius *A* will run, say, 10 motor tracts to the motor complex α ; to β will run, say, 6 directly anti-clockwise and one clockwise; to γ 3 anti-clockwise and 2 clockwise and so on. The maximal responses from the five muscles to stimulation of radial nerve *A* should then be in the following proportions: *A*, 10; *B* and *E*, 7; *C* and *D*, 5. If now a cut is made between *A* and *E* the expected responses will be *A*, 10; *B*, 6; *C*, 3; *D*, 2; and *E*, 1. Examination of Fig. 6*a* and *b* shows that this expectation is broadly confirmed. Thus when radial



(a)



(b)

Fig. 6. Records from a five-muscle preparation in which a supra-maximal stimulus is applied to each muscle in turn. Records below the traces indicate which radial nerve is stimulated. *a* with the radial nerve intact; *b* after the radial nerve has been severed between the muscles *E* and *A*. In all cases 10 stimuli at 2.5 s./sec. Intensity 10 V., duration 2 msec. Time marker 10 sec.

nerve *A* is stimulated, muscles *C* and *D* give weaker responses after section of the nerve ring. Similarly, when radial nerve *E* is stimulated the same effect can be seen with muscles *C* and *B*.

If such a picture is correct it will be expected that when the ring is intact evidence of the separate arrival of impulses which have passed clockwise and anti-clockwise around the ring should appear. This is occasionally observed in muscles which develop tension rapidly. The normal slow development of tension usually obscures the effect.

In the previous paragraphs it has been assumed that there is no convergence of the two sets of fibre tracts which supply the ultimate axons to produce the quick response or of those supplying the neurones within the motor complex to produce the delayed response. Thus, for example, it is assumed that the response from muscle *C* (Fig. 7) supplied by the motor complex γ will be proportional to the number of fibres entering the motor complex by clockwise and anti-clockwise paths. This assumption is not fully justified.

If, in a double-muscle preparation, as illustrated in Fig. 1, a supra-maximal stimulus is applied to radial nerve *E* and simultaneously a supra-maximal stimulus is applied to *E'*, the response from muscle *A* is no greater than when a single supra-maximal shock is applied to *E*. This effect is shown in Fig. 8. This suggests that the ipse-radial and para-radial fibre tracts do converge. With submaximal stimuli some summation occurs but the response to simultaneous stimulation is never greater, and usually is rather less than the algebraic sum of the separate responses to ipse-radial and para-radial nerve stimulation. These effects are found with both quick and delayed responses. The presence of such convergence necessitates some modification in detail to the system suggested tentatively in Fig. 7. It does not, however, offer an alternative explanation of the decremental effect since the latter persists after the nerve ring has been cut. Each motor complex then receives impulses from one set of fibres only and no convergence enters into the system.

An alternative explanation of the absence of complete algebraic summation when both nerves are stimulated simultaneously could lie in the presence of inhibitory nerves running from radial nerve *b* (Fig. 1) to the para-radial motor complex α and here partly inhibiting the effects of motor impulses arising from the radial nerve *a*.

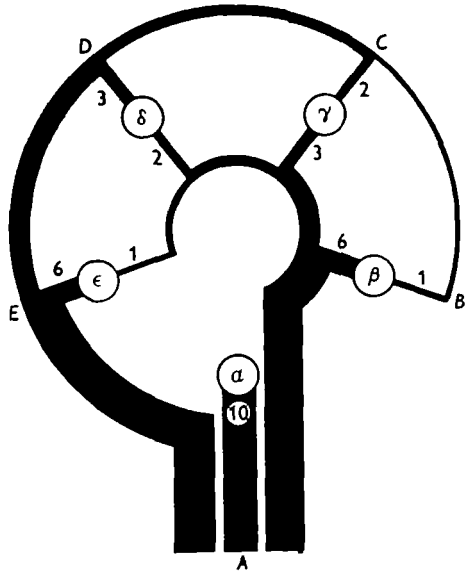


Fig. 7. Diagrammatic representation of the suggested arrangement of circumoral tracts arising from a single radius. The thickness of the lines is proportional to the number of nerve fibres. α , β , γ , etc., represent the motor complexes of the five pharyngeal retractor muscles. For further explanation see text.

This more complicated explanation cannot be excluded. It is found that if a stimulus is applied to *E'* and then subsequently to *E* the summed response from muscle *A* is no greater than when both nerves are stimulated simultaneously. Even when the interval between the two stimuli is as great as 10 sec. no difference in the magnitude of the summed responses is found. It seems then that if there are inhibitory nerves running between radii, their effect must persist for remarkably long times.

The 'decremental' effects shown in Figs. 2 and 6 might also be interpreted in terms of inhibitory nerves. The simplest relation would then be that from one radial nerve there ran to each motor complex in both clockwise and anti-clockwise directions equal numbers of motor nerves and varying numbers of inhibitory nerves. The inhibitory innervation pattern would then be the reverse of that suggested in Fig. 7. There is no evidence in favour of such a complex hypothesis, but

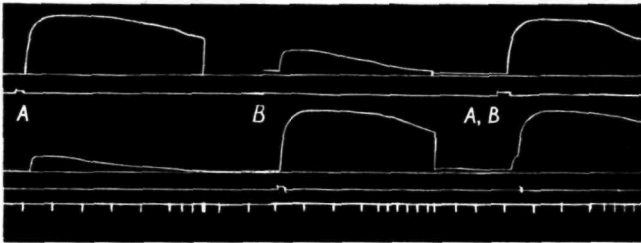


Fig. 8. Records from a double muscle preparation. A supra-maximal stimulus is applied first to one radial nerve, *A*, and then to the other *B*. Both radial nerves are then stimulated simultaneously, *AB*. Note that when both nerves are simultaneously stimulated the responses from the muscles are no greater than when only the radial nerve proper to the muscle is stimulated. In all cases 5 stimuli at a frequency of 2.5 s./sec. and intensity of 5 V. were applied. Time marker 10 sec.

if it be indeed the physiological basis of the observed decrement, its characteristic effects depend, as in the simpler explanation, upon the geometrical organization of fibre tracts and not upon some special physiological peculiarity of the synaptic structures.

One final point warrants comment. From a consideration of the dominance of different radii in the walking of a starfish, Smith (1945, 1950) suggests that the anatomical basis of the inter-radial circumoral tracts is such that from a single radius two tracts flow clockwise to the two left para-radii and two anti-clockwise to the two right para-radii. The circumoral connexions from any radius are organized like a horseshoe. However, the organization of the inter-radial tracts of *Cucumaria* which supply the retractor muscles is different since they run both clockwise and anti-clockwise to the most distal radius. There is of course no reason to assume that the two types of postulated organization are in any way mutually exclusive.

DISCUSSION

In general these experiments show that the characteristics of conduction around the circumoral nerve tracts supplying the pharyngeal retractor muscles of *Cucumaria* are very similar to those found in the radial nerve. The decremental conduction is, however, a distinct property for which no clear evidence was found in the radial

nerve. It is a known characteristic of echinoderm preparations (Smith, 1945) and has previously been explained in different ways.

Smith (1945, 1950) has suggested that the properties of the echinoderm epidermal network are similar to those of the nerve nets of sea anemones studied by Pantin (1935). Apparent decrement in anemones depends upon synaptic facilitation and is influenced by both the number and frequency of stimuli applied. We have found no evidence for such an effect in *Cucumaria*. All muscles of a five-muscle preparation respond to a single stimulus applied to a radial nerve and the characteristics of this response are not modified by repetitive stimulation in any manner suggesting the presence of frequency-facilitated synapses. These results with *Cucumaria* do not however exclude the possibility of such an effect in epidermal nerve nets.

Kinosita (1941) has studied the behaviour of sea-urchin spines and offers a different interpretation of decremental phenomena. He suggests that nerve tracts run out radially from the muscles around the base of each spine to the muscles of adjoining spines. There are usually no synaptic junctions between these different fibres. Propagated responses over the test depend upon proprioceptive relay mechanisms. If the muscles on one side of a spine are stimulated to contract, this movement stretches the muscles on the opposite surface of the spine. This stretch in turn stimulates nerve fibres running to the muscles at the bases of adjoining spines and the response thus spreads over the test. These proprioceptive links do not respond in an all-or-nothing manner and this is regarded by Kinosita as the origin of diffuse and decremental responses.

Translated into terms of the retractor muscle system of *Cucumaria* this would imply that relay around the circumoral ring would depend upon proprioceptive links between succeeding retractor muscles. No evidence of such links has been found. In a five-muscle preparation neither the stretching of a single muscle nor direct electrical stimulation applied to a single muscle causing it to contract produces any response from the other four muscles. Furthermore, as shown in Fig. 5, excitation will pass unchanged beyond a radius in which the response of the muscle has been almost completely eliminated by destruction of its motor nerve and motor complex. Clearly the system envisaged by Kinosita does not account for the decremental conduction here observed. This, of course, does not exclude the possibility of a different mechanism occurring in the epidermal muscular systems of echinoids.

Our results suggest that the decremental effects in circumoral conduction which we have observed are due simply to the spatial organization of nerve fibre tracts. The general similarity between the properties of the circumoral and radial nerves supports Smith's (1950) suggestion that the circumoral tracts serve mainly to connect the radii and correspond in no way to a 'central nervous system'. The anatomical basis of 'central nervous' functions in an echinoderm probably lies in its numerous and scattered motor complexes.

SUMMARY

1. The general characteristics of circumoral nervous conduction in *Cucumaria* have been studied by the use of preparations consisting of the retractor muscles and radial nerves of two adjoining radii joined by a sector of circumoral nerve ring and

by the use of similar preparations of all five retractor muscles and the complete circumoral nerve ring.

2. The characteristics of the responses of muscles stimulated by way of circumoral nerve tracts are as follows: the muscles respond with a quick and a delayed response; the magnitude of these responses depends upon the intensity of stimulation applied to an adjoining radial nerve, but is unaffected by frequency of stimulation up to a rate of 10 s./sec.; at high frequencies of stimulation both quick and delayed responses are depressed; the conduction velocity of impulses releasing quick and delayed responses is of the same order; the delayed response may show a prolonged facilitation previously analogized with post-tetanic potentiation. In these characteristics the muscular responses to impulses conducted in the circumoral nerve tracts are similar to those found to impulses conducted in the radial nerve tracts alone.

3. When, in a preparation of the five-retractor muscles, a radial nerve is stimulated, the muscles of radii nearer the stimulated nerve contract more strongly than those of radii further away.

4. Evidence is presented in favour of the view that this 'decremental' effect is dependent upon the geometrical arrangement of the fibre tracts in the circumoral nerve. The effect is not dependent upon frequency-sensitive synaptic junctions nor upon proprioceptive relays.

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Note added in proof. Shortly after this paper went to press an important study by G. A. Kerkut (The mechanisms of co-ordination of the starfish tube feet. *Behaviour* (1954), **6**, 206-32) appeared. In this evidence is presented that, contrary to Smith's (1945, 1950) interpretation, in the starfish excitation can pass from one radius around the circumoral nerve ring to the most distal radius. This agrees with our findings in *Cucumaria*.