

THE ELECTRICAL ACTIVITY OF THE RADIAL NERVE IN *DIADEMA ANTILLARUM* PHILIPPI AND CERTAIN OTHER ECHINOIDS

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INTRODUCTION

Electrophysiological studies in echinoderms are rare. In echinoids investigations have been made by Takahashi and Sandeman. The former used *Diadema setosum*, the latter *Strongylocentrotus*, *Tripneustes* and *Toxopneustes*. Takahashi gives a brief account of the electrical concomitant of the photic responses already described in detail by Millott and co-workers. Behavioural studies showed that the radial nerves had certain properties of a central nervous system, in particular that they functioned as centres for interaction between excitation and inhibition. The aim of this account is to show some of the electrical manifestations of such activity.

METHODS

Although most attention was devoted to the radial nerves of *Diadema*, the responses of these nerves were also examined in *Arbacia lixula* (Linn.), *Echinus esculentus* Linnaeus and *Paracentrotus lividus* (Lamarck).

Two types of preparation were used. In one the radial nerve remained largely intact, attached by its side branches to the adjoining body-wall with its effectors. A short length at the oral end, freed to allow stimulation and recording was lifted on to electrodes immersed in liquid paraffin. The remainder of the nerve was immersed in natural or artificial sea water (Harvey, 1945). More often the nerve was excised and covered by liquid paraffin.

Both stimulating and recording electrodes rested on the surface of the nerve and were either of platinum or silver-silver chloride. Recording electrodes, spaced at 4.0 mm. were resistance-capacity coupled to a high-gain a.c. pre-amplifier feeding a 'Tektronix' oscilloscope type 502A. Stimuli in the form of square pulses from a Grass Stimulator type S.D.5, were given by electrodes, the nearer of which was placed 5.0 mm. from the recording electrodes.

In some cases spine movements were simultaneously recorded by allowing a selected spine to intercept a light beam directed on to a phototransistor.

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RESPONSES OF THE RADIAL NERVE TO ELECTRICAL STIMULATION

Single stimuli

Pulses of 1–10 V. and 0.5–5.0 msec. produced responses that differed in the species of urchin examined.

In *Arbacia*, *Echinus* and *Paracentrotus* a simple diphasic response is observed lasting for 140–200 msec. (Fig. 1 A–C). Though the amplitude varies considerably with the intensity and duration of the stimulus, the duration of the response varies little.

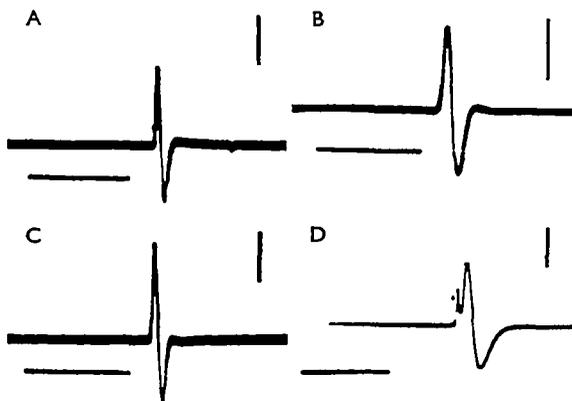


Fig. 1. Electrical responses of the radial nerves to a single stimulus of 10.0 V., 2 msec. A, *Arbacia*; B, *Echinus*; C, *Paracentrotus*; D, *Diadema*. Horizontal scales, in A, B and C = 1.0 sec; D = 250 msec. Vertical scales in all cases = 200 μ V.



Fig. 2. *Diadema*: the effect of 0.1% 'Procaine'. A, Normal response to stimulus of 8.0 V., 1.8 msec.; B, response to same stimulus after radial nerve had been immersed in 'Procaine' for 40 min. Scale = 400 msec.

The response of *Diadema* shows two distinct peaks (Fig. 1 D) of which the first is usually smaller in amplitude and always shorter in duration.

That these responses are propagated disturbances in nerve and not due to spread of electrical charges generated in other active tissue such as muscle is shown by several considerations. Thus blocking conduction by crushing the nerve, or by immersing it in 0.1–3.0% 'Procaine' or in calcium-free sea water, in the region between stimulating and recording electrodes, abolishes the response (Fig. 2). After such treatment by immersion, normal excitability returned when the nerve was replaced in sea water.

That the electrical charges are propagated actively rather than spread passively is shown by the size and time relations of the stimulus and response. It is also shown

by reversing the electrodes. When the negative electrode is nearer the recording electrodes an initial depolarizing phase appears in the response. When the positive electrode is nearer, the response is not merely reversed in polarity, it is altered in character. This is especially striking if d.c., or repetitive stimulation is used (Fig. 3). Although the initial phase is now hyperpolarizing, depolarization is abolished, which suggests the occurrence of a depressing effect on the excitability of a living membrane. This inference is supported by the evidence of refractory states (see below). That the effect is not purely electrotonic is also shown by chilling the nerve to 3° C., which markedly reduces the electrical changes recorded.



Fig. 3. *Echinus*: the effect of reversing the polarity of the stimulating electrodes when a 2.0 V. d.c. current was used. A, Response with negative stimulating electrode proximal to recording electrodes; B, response with position of stimulating electrodes reversed. Scale = 1.0 sec.

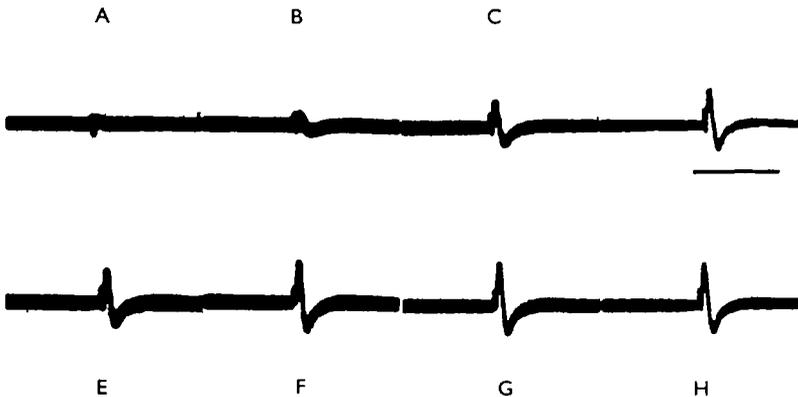


Fig. 4. *Diadema*: the effect of increasing the intensity of stimulation. In each instance the duration of the stimulus was 2.0 msec. Intensity of the stimulus in A, 2.0 V.; B, 4.0 V.; C, 6.0 V.; D, 8.0 V.; E, 10.0 V.; F, 20.0 V.; G, 40.0 V.; H, 60.0 V. Note attainment of maximum amplitude in F, beyond which there is no further increase. Scale = 1.0 sec.

The relation between the amplitude of the response and the strength of stimulation is also important. Responses, whether of the single or double-peaked type, increase in size with increasing stimulation, up to a defined limit (Fig. 4).

Taken collectively the evidence indicates the gross activity of a nerve with many constituent fibres. This is supported by evidence from electron microscopy obtained by Cobb & Laverack (1966) and Millott & Okumura, (unpublished), who showed the existence of large numbers of minute fibres in the radial nerves of *Echinus* and *Diadema* respectively.

Conduction of the electrical responses in the radial nerve, whether orally or aborally, is accompanied by a marked decrement in amplitude (Fig. 5). With the greatest amplification available it was only just possible to distinguish a response

from accompanying 'noise' at a distance of 2.0 cm. from the nearer stimulating electrode, whether it be oral or aboral in position. The cause of the decrement is not known, it could well be due, at least in part, to synaptic loss.

The effect of cutting partially across the radial nerve in between stimulating and recording electrodes diminishes the amplitude of the propagated potentials, but their form remains unaltered. This could be due to the lesser number of nerve fibres participating, provided they are firing more or less in phase. This is possible, bearing in mind the short distance between stimulating and recording electrodes. Nevertheless, such a finding differs from that reported by Sandeman (1965) who found that splitting the radial nerve lengthwise sometimes increased the potentials as well as altering their form. Again, we have as yet found no evidence of the tracts reported by Sandeman, because in our experiments short cuts through both central and peripheral regions of the radial nerve exert similar diminishing effects on the recorded responses.

The double-peaked response of *Diadema* merits closer examination. Important evidence is forthcoming from the effect of repetitive stimulation.

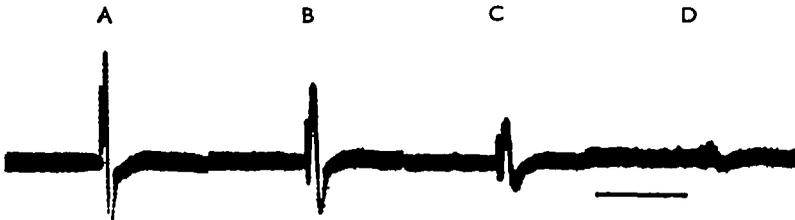


Fig. 5. *Diadema*: manifestation of a decrement. The responses from left to right were recorded at increasing distances from the stimulating electrodes. A, 2.0 mm.; B, 4.0 mm.; C, 6.0 mm.; D, 8.0 mm. Stimulus 10.0 V., 2.0 msec. Scale = 1.0 sec.

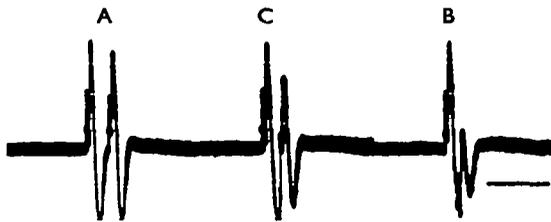


Fig. 6. *Diadema*: responses to two stimuli separated by varying intervals. Responses from left to right are to stimuli of 2.0 V., 2 msec., separated by 200, 120 and 80 msec. Note diminution of the responses to the second stimulus. Scale = 500 msec.

Repetitive stimuli

The effect of repetitive stimulation varies with frequency. In all cases it can excite more than one response of the same kind, but not necessarily of the same amplitude. Stimuli separated by more than 200 msec. excite a second response equal in size to the first, except in *Paracentrotus* where the second stimulus excites a smaller response unless given more than 1 sec. after the first. More frequent stimulation excites progressively smaller responses (Fig. 6) in all the urchins studied and if the frequency exceeds 12 to 17 stimuli per second, no response appears, after the first. This is interpreted as the consequence of relative and absolute refractory periods. Rough estimates of their length can be made by observing the effect of increasing pro-

gressively the frequency of stimulation, using both pairs and trains of stimuli. The range of intervals between stimuli after which the second or successive responses diminish, indicates the duration of the relative refractory period. Similarly, the range over which the second response fails to appear indicates the duration of the absolute refractory period.

If the frequency is raised to between 25 and 150 cyc./sec., a point is reached, depending on the species concerned, at which a response re-appears in augmented form. This could be due to latent addition occurring during the period preceding the absolute refractory state. Estimates of the length of these periods made in this way are shown in Table 1. They have only limited value, for the responses on which they are based represent massed activities of nerve elements which may differ considerably in excitability and conduction velocity.

Table 1. *Characteristics of the gross potentials in the radial nerves*

	Conduction velocity (cm/sec)		Period of latent addition (msec.)	Absolute refractory period (msec.)	Relative refractory period (msec.)
	Method I	Method II			
<i>Arbacia</i>	5.4-6.9	6.0-8.0	10	10-60	60-200
<i>Diadema</i> { 1st	18.1-27.0	22.0-33.0	4	4-20	20-70
{ 2nd	5.0-6.9	5.5-8.0	10	10-60	60-200
<i>Echimus</i>	3.6-6.5	4.5-8.0	40	40-80	80-200
<i>Paracentrotus</i>	5.7-8.1	6.5-9.0	10	10-60	60-1100

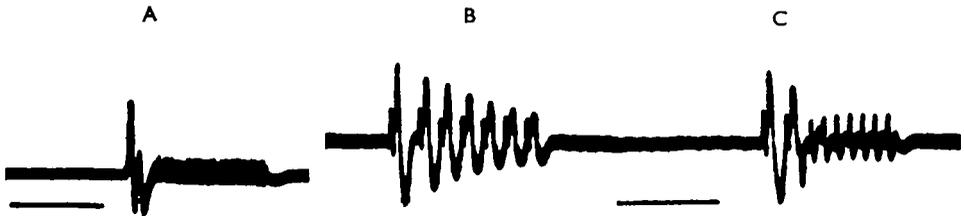


Fig. 7. Responses to repetitive stimulation. Scale = 1.0 sec. A, *Paracentrotus*: response to 8.0 V., 1 msec. stimuli at 200 cyc./sec. Note the appearance of a second peak. B, *Diadema*: responses to 10.0 V., 1 msec. stimuli at 6 cyc./sec. Note the diminution of the second peak. C, *Diadema*: responses to the same stimuli as in B, but at 10 cyc./sec. Note reduction in the number of second peaks.

Two effects of repetitive stimulation deserve special mention. Stimulation at 200 cyc./sec. produced a singular effect in *Paracentrotus*, where on two occasions it produced a second peak as shown in Fig. 7A. We have not investigated its significance. In *Diadema* repetitive stimuli exert a revealing effect in separating the two peaks of the response already reported.

Significance of the double peaks in Diadema

Stimuli at 6 cyc./sec. cause a gradual diminution in amplitude of the second of the two peaks but not in that of the first (Fig. 7B). This suggests that the peaks represent the responses of groups of fibres with differing refractoriness. The idea is strengthened by the effect of stimulating at 10 cyc./sec., which reduces the number of second peaks to one or two, leaving that of the first unaffected (Fig. 7C). The earlier of the

two peaks persists up to frequencies of over 40 cyc. These findings indicate that the two peaks are responses in fibres having absolute and relative refractory periods with the approximate durations shown in Table 1.

The differing character of the responses is confirmed by their different thresholds (Fig. 8), for with stimuli of 1.8 msec., as the intensity is increased from very small values, the first response appears alone at 2 V. The second response does not appear until the stimulus reaches 4 V., when it is much smaller than the first. Above 4 V. the size of the second response rapidly transcends that of the first.

Further indications that the two peaks represent differing responses are obtained from the effects of cooling and anaesthetics on the radial nerve. Cooling to 3° C. for 2 hr. abolishes the second peak, but merely reduces the size of the first. Again, 3.0% 'Procaine' solution abolishes the second peak in 5 min., whereas the first persists for 35 min.

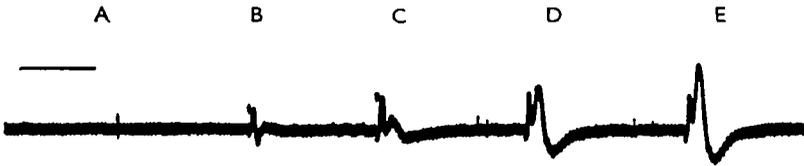


Fig. 8. *Diadema*: thresholds of the first (fast) and second (slow) responses. Responses to stimuli of 1.8 msec. at increasing voltages from left to right. A, 1.0 V.; note absence of response, the vertical line is the stimulus artifact. B, 2.0 V.; note appearance of first response. C, 4.0 V.; note appearance of second response. D, 6.0 V. E, 8.0 V.; note increase in size of the second response. Scale = 500 msec.

CONDUCTION VELOCITY

This was estimated by two methods. In one (Method I), the time interval between maximum positive and negative deflexions of a single response was measured. This gives the time required for the disturbance to pass over the known distance between the recording electrodes. In the other (Method II), the time interval between stimulation and the beginning of a response was measured. This includes the time required for the response to appear as well as that required for it to pass between the stimulating and recording electrodes. An estimate of the conduction velocity can be obtained by subtracting, from the total time, the duration of the period preceding the absolute refractory period as given in Table 1. The data obtained from both methods are in reasonable agreement as shown in Table 1.

It will be seen that in *Diadema* the two peaks are conducted with differing speeds, so that the first and second may be distinguished as fast and slow responses.

ORAL AND ABORAL CONDUCTION IN *DIADEMA*

The foregoing estimates of conduction velocity make it possible to show that oral and aboral conduction occurs in separate nerves.

When a radial nerve is stimulated electrically at both ends and recording electrodes are placed between the stimulating electrodes, the effect of potentials travelling in opposite directions can be observed. If the recording electrodes are sited 4.0 mm. away from the stimulating electrodes on either side and if the stimuli at the two ends are not applied simultaneously, but separated by 40–200 msec., two discrete

electrical responses can be recorded. Because seven electrodes were employed on a short length of nerve, the high level of 'noise' implied that only the second (slow) peak was distinguishable at the stimulus intensities used. The conduction velocity of the slow response started at one end implies that it will reach the recording electrodes during the absolute refractory period resulting from the slow response to an earlier stimulus imparted to the other end. Figure 9 shows that two such stimuli separated by 100 msec., elicit responses that pass through a region that is estimated to be absolutely refractory, producing the potentials recorded. This could occur only if the observed potentials were passing in opposite directions along separate pathways.

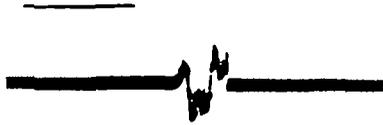


Fig. 9. *Diadema*: demonstration of separate paths of oral and aboral conduction (see p. 285). One stimulus of 8.0 V., 2.0 msec. was given to each end of the radial nerve, separated by an interval of 100 msec. Note the appearance of two second (slow) responses. Scale = 500 msec.

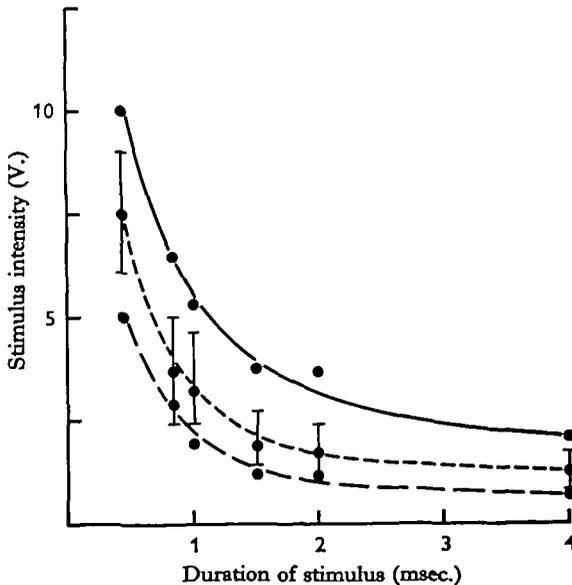


Fig. 10. *Diadema*: strength-duration relationships of the first (slow) and second (fast) electrical responses compared with those of spine movement (see p. 286). Abscissae: duration of stimulus in msec. Ordinates: intensity of stimulus in volts. A (solid line), slow response; B (long dashes), fast response; C (short dashes), spine movement. Vertical lines represent the range in stimulus intensity required to elicit the smallest visible spine response in twelve experiments.

THE FUNCTION OF THE FAST RESPONSE IN *DIADEMA*

The importance of the radial nerves of sea urchins as centres involved in spin movement is well known (Millott & Takahashi, 1963; Bullock, 1965; Smith, 1965) The present investigation confirms their importance in this connexion, for stimulation of the radial nerve *in situ* was often followed by spine movement. This makes it

possible to examine the relation to spine movement of the two potentials observed in *Diadema*.

A significant indication is obtained by comparing the strength-duration relationships of the electrical responses in the radial nerve and the similar relationships of the spine-muscle contractions that ensue. In these experiments a piece of body wall with its spines was used, the concavity of the test being filled with liquid paraffin to avoid direct spread of current from the electrodes on the radial nerve. The relationships were obtained by varying independently the strength and duration of the stimulus so as to elicit a standard (in this case, threshold) response at the site of the recording electrodes and in an adjacent spine. The relevant curves are shown in Fig. 10. It will be evident that the strength-duration relationships of the spine movements observed correspond with those of the fast potential and not with those of the slow.

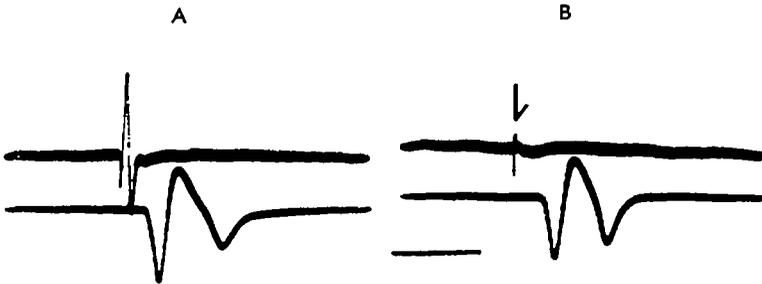


Fig. 11 A, B. *Diadema*: the relation between the first (fast) electrical response (upper trace) and spine movement (lower trace) recorded by a phototransistor as discussed on p. 286. In A, note the presence of both fast and slow responses. In B, where a weaker stimulus was used, the fast response alone persists (at arrow). Scale = 1.0 sec.

It can also be shown that the slow potential is not directly concerned in exciting spine movement by chilling the radial nerve to 3° C. for 20 min., which eliminates the slow potential but not the fast. Spine movements continue in response to electrical stimulation of the chilled radial nerve.

A more direct indication of the function of the fast potential is obtained by using the different thresholds of the two electrical responses. Stimuli which are sub-threshold for the slow response will continue to excite the fast, and at the same time elicit spine contractions as shown by the simultaneous recordings in Fig. 11. Conversely, selective elimination of the fast electrical response, by reversing the stimulating electrodes so that the positive lies nearer the recording electrodes, likewise eliminates the excitation of spine movement.

INTERACTION OF RESPONSES TO ELECTRICAL AND PHOTIC STIMULATION; THE SIGNIFICANCE OF THE SLOW RESPONSE

The significance of the slow electrical response in *Diadema* is revealed by its effect on the responses to photic stimuli. Such stimuli, provided by light spots 2.0 mm in diameter projected on the radial nerve in the manner already described (Yoshida & Millott, 1959), elicit protracted spine oscillations when the light is extinguished. These reactions are markedly inhibited by single, or repetitive electrical stimuli

given to the radial nerve immediately before shading, or after it, during the latent period of the shadow response, or after the ensuing spine movements have begun. It is not possible, however, to inhibit the electrical responses by photic stimuli.

According to the intensity, duration and number of electrical stimuli given, the photic response may be inhibited completely (Fig. 12B) or partially, in which case

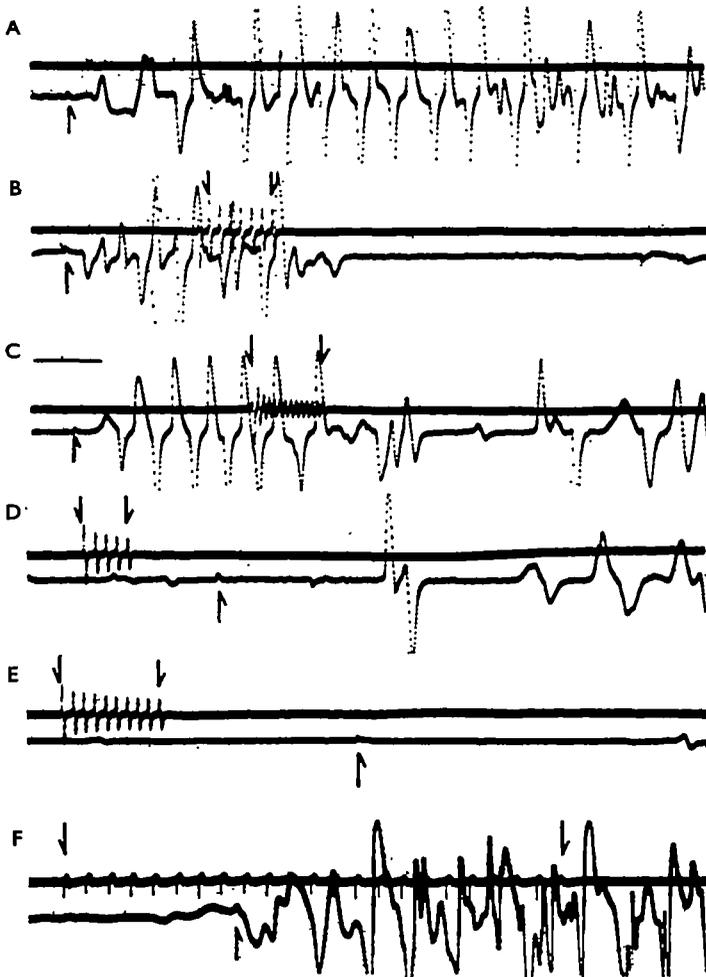


Fig. 12. *Diadema*: inhibition of the shadow response by the slow electrical potential (see pp. 287-8). In each case obliteration of the light spot on the radial nerve occurred at the lower arrow. In B-F, the upper traces show the electrical responses (between upper arrows), the lower traces show the responses of a single spine. Scale = 1.0 sec. A, Oscillation of a single spine produced by shading. B, Complete inhibition of the shadow response produced by repetitive electrical stimulation of the radial nerve. C, Partial inhibition produced by a similar stimulus. Note the reduction in amplitude, frequency and regularity of the spine movements. D, E, The relation between stimulus and the duration of inhibition. In D stimulation by five pulses produces a relatively short inhibition, so that a response follows shading at the lower arrow. In E, ten pulses of the same size inhibit for longer, so that a clear response to shading fails to appear. F, Lack of any clear inhibitory effect following repetitive electrical stimulation that is subthreshold for the slow potential. Note only the fast potential appears between the upper arrows.

the spine oscillations are reduced in amplitude, frequency, duration and regularity (Fig. 12C). Again, the duration of inhibition is a function of the strength of the electrical stimuli (Fig. 12D, E). Complete inhibition requires four or more stimuli per second, each of sufficient strength to elicit a maximal electrical response.

In view of the differing thresholds of the fast and slow responses (p. 284) and the size of the stimuli required to achieve inhibition, it is evident that the slow and not the fast potentials are concerned. This is confirmed by the inability of the fast potentials alone to exert any clear inhibitory effect on the photic response (Fig. 12F).

DISCUSSION

In view of the complexity of the radial nerves and the fact that external electrodes only were used, records comparable with those reported by Takahashi (1964) following photic stimulation, were not obtained. The foregoing observations on massed potentials are to some extent comparable with those reported by Sandeman (1965) from other species, but detailed comparisons would be unprofitable because the responses recorded are necessarily conditioned by the distance between the electrodes and this is not always specified by Sandeman.

In *Arbacia*, *Echinus* and *Paracentrotus* the electrical responses to single stimuli lack the complexity of those reported by Sandeman, which in some respects resemble those we have described in *Diadema*. However, the two peaks seen in the responses of *Diadema* do not overlap, and they represent separate responses differing considerably in conduction velocity as well as in other respects, so that they can readily be separated. The conditions which Sandeman envisages as a possibility are therefore realized in *Diadema*.

The existence of decremental conduction in the radial nerves, reported by Sandeman, suggests the occurrence of synapses along the course of the pathways in these nerves. This is substantiated by our findings. It also agrees with what we have found in studying the fine structure of the radial nerves in *Diadema* (N. Millott & H. Okumura, unpublished). Although there are clear signs that conduction of excitation in oral and aboral directions occurs in different pathways, we obtained no evidence indicating that the potentials responsible in the two cases are restricted to localized definable fibre tracts in the radial nerve.

The most interesting and significant aspect of our findings is the relation between the recorded potentials and the animal's activity. Sandeman suspected that the potentials in the radial nerves were related to ampullary activity. We have not investigated this possibility, but our findings show clearly that the two potentials recorded in *Diadema* are related to spine movement.

Diadema is remarkable among sea urchins in its striking manifestation of photosensitivity in the form of spine reactions. An analysis of these has shown that light can inhibit as well as excite spine movements. From this it was inferred that the intimate mechanism of the shadow response involved interplay between excitation and inhibition within and outside the radial nerve (Millott & Yoshida, 1960a, b; Yoshida, 1966). This was substantiated by Takahashi's (1964) skilful electrophysiological demonstration of 'on' and 'off' effects following photic stimulation of the radial nerve. The direct demonstration of propagated potentials in the radial nerve which

are in some way related to the inhibition of spine movements excited photically, provides further evidence for this view.

It should be noted, however, that although the fast potentials are related to the excitation of spine movements, these differ from the movements excited by photic stimuli, being much slower and more irregular than those characteristic of the shadow response.

SUMMARY

1. The propagated massed potentials which follow stimulation of the radial nerve in *Arbacia*, *Diadema*, *Echinus* and *Paracentrotus* are described.

2. Approximate values for the averaged absolute and relative refractory periods and the conduction velocity were obtained.

3. The response of *Diadema* has a double peak which is shown to represent responses of nerves differing in excitability and conduction velocity. The fast potential is concerned with spine movement. The slow potential is related to inhibition of spine movements excited photically.

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