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The Shadow Reaction of Diadema antillarum Philippi. IV. Spine Movements and their Implications

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THE SHADOW REACTION OF *DIADEMA ANTILLARUM* PHILIPPI

IV.* SPINE MOVEMENTS AND THEIR IMPLICATIONS

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[Plates 52 and 53]

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The aboral primary spines of the echinoid *Diadema antillarum* react to localized shadows by a movement that is frequently constant in direction, followed by continued oscillation. Both direction and the degree of constancy are determined by the positions, relative to the spine, of the stimulating light (surface factor), oral pole (oral directing factor) and radial nerve (ambulacral directing factor). The oral and ambulacral factors appear to be an expression of the route by which nervous excitation reaches the spine from the radial nerve. The surface factor does not involve the radial nerve and appears to interact with the other factors at the spine base.

Transmission of excitation over the skin can occur at differing speeds, suggesting that there are at least two divisions of the superficial nervous system, which has characters inappropriate to an anastomosing network. There is evidence that transmission does not always depend on proprioceptive relays at the spine base.

The area of influence of a radial nerve extends at least half-way around the test. The frequency with which the spines oscillate is governed, at least in part, by excitation from these nerves. This effect, which is decremental, together with the propensity of the radial nerves to generate oscillations of the spines by spontaneous discharge and their capacity to serve as centres of interaction between excitation and inhibition, suggest that the nerves are more than simple local reflex centres. Their properties resemble those of a more elaborate central nervous system.

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Introduction

It has long been known that the spines of echinoids respond to various kinds of stimuli. Particular attention has recently been paid to the spine responses of *Diadema*, by Millott (1954), Millott & Yoshida (1960*a*, *b*), Yoshida & Millott (1959, 1960) and Yoshida (1963), who have studied the reaction that ensues when a light spot, projected on to the skin, is obliterated. The animal responds by waving its spines and although the reaction follows extinction of the light, there is good reason to think that the light, and not the shading, constitutes the stimulus (Millott 1960).

The character of the movement is related to the stimulus in a fairly precise way. The amplitude of the swing, its frequency and the time for which the waving persists, vary with the intensity and duration of the lighting (Millott & Yoshida 1960*a*). But there is another feature of the shadow reaction which has received insufficient attention, namely, the direction of the first movement of the spine, which is often related to the location of the stimulus. Von Uexküll (1900*a*), Cowles (1911) and Dahlgren (1916), record this important feature of the response and a fuller description is given by Millott (1954), but all these accounts, based on direct observations, mostly of whole animals, are inadequate.

The simultaneous responses of many spines are not easily resolved, but the methods of recording spine movement and localizing light stimuli, developed by Millott & Yoshida (1957), and Yoshida & Millott (1959), have made possible a more adequate analysis.

Although the first movement of a spine is frequently made toward the area of the animal's surface that has been shaded, it is not always so, and further analysis soon makes it clear that the situation is more complex and that several factors are involved.

The direction in which spines move has wider implications in so far as it has been generally held, following von Uexküll (1900*a*) and Kinosita (1941), that it corresponds to the direction from which excitation approaches them. If this be true, then deductions might be made concerning the pathways of excitation in the extensive and diffuse superficial nervous system of echinoids, concerning which little is known. Again, studying the direction of these movements affords an opportunity to examine, critically, another aspect of this general view, namely, that the nervous events determining direction in localized spine responses are confined to the superficial nerve layer (Hyman 1955).

Finally, the protracted, rapid beating of the spines seen in the shadow response of *Diadema*, is characteristic of responses to light stimuli and distinguishes them from reactions to stimuli of other kinds. This distinction has been insufficiently appreciated and deserves further study in relation to the nervous organization.

Methods

These were essentially similar to those described in a preceding account (Millott & Yoshida 1960*a*). The preparations used, however, were sometimes larger pieces of test, including more than one ambulacrum and radial nerve, and the intervening interambulacra. In most cases the regions of the peristome and periproct were removed, together with the remains of any nerve ring. In a few specified cases a portion of the circumoral nerve ring was retained. Most of the spines were removed, leaving between two and four primary spines intact. Unless otherwise indicated, the spine shaft was removed, leaving the base undamaged. The movement of the intact spines was recorded photographically and in most cases the record was correlated with direct observation. Although attention has been concentrated on photic responses, some has been paid to responses to electrical or contact stimuli and to spontaneous movements.

With photic stimulation only shadow responses were studied, preparations being illuminated for 3 min and then shaded by the action of an electromagnetic shutter which cut off the light completely for several minutes while the response was in progress. The stimulating light was the same as that used previously (Millott & Yoshida 1960*a*) and run on direct current only. The light beam was concentrated to form stimulating spots of various sizes, by means of suitable lenses incorporated in a system essentially similar to that previously described (Yoshida & Millott 1959).

A number of special techniques were used, which will be described later in conjunction with the experiments.

THE DIRECTION OF THE FIRST MOVEMENT

1. *Factors influencing direction*

Observations have been almost entirely confined to the primary spines within and immediately alongside the ambulacra (*A.s.*, *I.s.*, figures 4 and 5, plate 52), but there are marked differences between their responses. The spines of the oral region (*O.s.*, figure 5) show little if any response to photic stimuli, so that the responsive area of a meridian extends along about three-quarters of its length from the aboral pole (*Ap.*, figure 5). The spines below the ambitus (E, E', figures 4 and 5) respond slowly, feebly and inconstantly as compared with their aboral counterparts. (Plate 52 faces p. 468.)

Although in the shadow response, the spines may make their first movement toward the position of the light spot that has been obliterated, the movement is rarely direct, more often it is oblique, which in itself indicates that more than one directing factor is operative. Without elaborate photographic apparatus it is not possible to record the direction of such movements accurately, but for the purpose on hand it is adequate to consider them as made with reference to some convenient, fixed foci, irrespective of whether their movement, towards or away from these areas is direct or oblique. Movement will therefore be described with reference, not only to the light spot, but also to the oral and aboral poles (figure 4), between which it will be termed meridional, and in relation to an ambulacrum (figure 5), movement towards or away from which will be described as lateral.

A simple way of indicating consistency of direction is by the ratio of the number of first movements made towards a given position (n) to the total number of first movements observed (N). The ratio n/N will be termed the *directional index*, so that an index of unity implies that every first movement has at least one component directed towards the same area, whereas an index of 0·5 implies that such movements have no consistent direction with respect to the area.

The deterioration of the preparations used made it impossible to make large numbers of records of the movements of one particular spine, so that similar spines from several preparations had often to be used. To indicate this the number of preparations used is shown in each of the succeeding tables of results, in parentheses after the values for N. The procedure appears acceptable because the directional indices for similar preparations, treated in the same way, were found to be of the same order, so that differences between preparations can largely be discounted.

When a light spot is projected on to an area of the skin that is oral with respect to an interambulacral spine, the first movement of the spine is almost invariably directed orally, but if it is projected on to an area aboral to the spine, the direction of the first movement is erratic, being aboral in half of the trials, and oral in the remainder (table 1).

TABLE 1. MERIDIONAL MOVEMENTS

In this and the following tables figures in parentheses represent the numbers of preparations used.

position of spine	position of light spot	number of trials (N)	number of movements towards light spot (n)	directional index (n/N)
interambulacrum	aboral to spine	60 (7)	30	0·50
interambulacrum	oral to spine	80 (7)	74	0·93
ambulacrum	aboral to spine	27 (5)	4	0·15
ambulacrum	oral to spine	27 (5)	27	1·00

Ambulacral spines behave similarly except that their first movement is more consistently oralwards (table 1).

The movements of both kinds of spine are therefore biased, but their tendency to point oralwards is disturbed, though to differing degrees, by an aboral light spot.

If light spots are projected in a latitudinal plane on to the skin covering the test on either side of an interambulacral spine, a somewhat similar phenomenon is observed. When the light spot falls on the adjoining ambulacrum (*A*, figure 1), the spine usually directs its first movement towards this side (figure 1 and table 2). When the light spot is shifted, so that it falls on the opposite side of the spine (*B*, figure 1), the direction of the first movement is changed, so that the spine points to the light spot in its new position, but less consistently than before (figure 1 and table 2).

Again, spines are influenced by the position of the light spot (and to a greater degree), but the effect of the light spot is greater when it falls on the same side of the spine as the ambulacrum through which the reflex is consummated, so that the spine appears to be biased toward the operative ambulacrum.

The behaviour of the ambulacral spines is different. When the light spot is projected on to the centre of their own ambulacrum (*A*, figure 1), the spines arising from either side of the ambulacrum make their first movement towards it, but less consistently than in the case of the interambulacral spines (figure 1 and table 2). When the light spot is shifted on to the adjoining interambulacrum (*B*, figure 1), the spines arising from both sides of the ambulacrum point towards it with substantially improved consistency. If the light spot is displaced still further, so as to fall on the next ambulacrum (*C*, figure 1), movement towards it is still more consistent (table 2).

The direction of the first movement of both ambulacral and interambulacral spines is influenced by the position of the light spot and the operative ambulacrum, but an important difference emerges. Thus with the ambulacral spines, displacement of the light spot from their own ambulacrum to the adjoining interambulacrum does not diminish, but improves the degree of directional consistency, despite the fact that in the case of one of the spines the light spot has been shifted so as to lie on the side of the spine opposite to the ambulacrum. The proximal ambulacrum therefore seems to play little part in determining the direction of lateral movements of the spines arising from it.

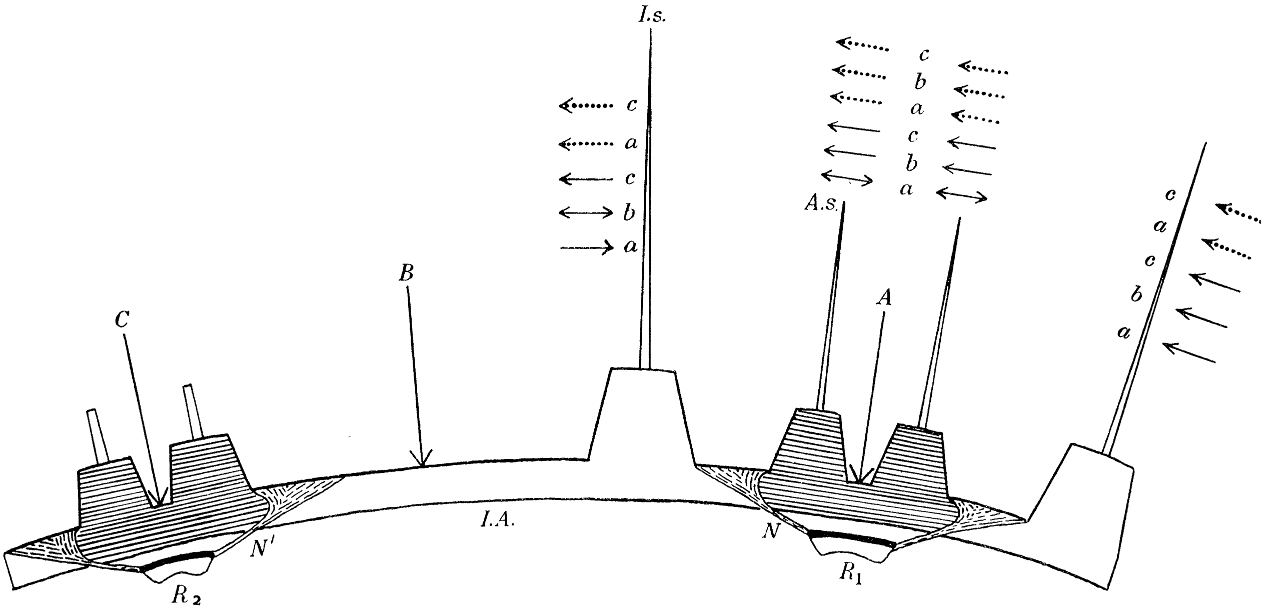

FIGURE 1. Lateral spine movements (pp. 440 to 442). A diagrammatic transverse section of portion of the test, consisting of an interambulacrum (*I.A.*), flanked by two ambulacra (shaded) with their radial nerves (R_1 and R_2). Only the spines, the movements of which are discussed, are shown complete; the remainder are cut short or omitted. The diagram is distorted, since not all of the structures would appear as shown, in the same latitudinal plane of section. The positions of the light spots referred to on p. 440 are shown by *A*, *B* and *C*, and the direction of the first movement made by each of four spines (two ambulacral and two interambulacral), following the extinction of these light spots, is shown by the arrows labelled by corresponding letters, placed alongside each spine. Solid arrows indicate the direction of the first movement when both radial nerves are present; dotted arrows indicate the direction when R_1 has been removed. Double-headed arrows indicate a first movement that is inconsistent in direction. *N*, *N′*, branches of radial nerve. *A.s.*, ambulacral spine. *I.s.*, interambulacral spine.

TABLE 2. LATERAL MOVEMENTS

position of spine	position of light spot	number of trials (*N*)	number of movements towards light spot (*n*)	directional index (*n*/*N*)
interambulacrum	proximal ambulacrum	84 (13)	70	0·83
interambulacrum	same interambulacrum, abambulacral to spine	79 (8)	51	0·65
interambulacrum	distal ambulacrum	45 (8)	38	0·84
ambulacrum	centre of same ambulacrum	58 (7)	39	0·67
ambulacrum	adjoining interambulacrum	34 (5) *a* 18 *b* 16	27 *a* 14 *b* 13	0·79 *a* 0·78 *b* 0·81
ambulacrum	neighbouring ambulacrum	48 (8) *a* 28 *b* 20	45 *a* 26 *b* 19	0·94 *a* 0·93 *b* 0·95

a, spine on the side of the ambulacrum nearer to the light spot.
b, spine on the side of the ambulacrum more removed from the light spot.

Three factors, therefore, appear for the moment to influence the direction of spine movement: the position of the light spot, which may be termed the surface factor, an ambulacral directing and an oral directing factor. The relative importance and the interaction between these factors will emerge as other features of the responses are studied.

With the ambulacral spines, the relatively feeble directional effect of stimuli delivered to their own ambulacrum suggests a weak ambulacral factor. This could account for their behaviour, for its opposition to the attraction of an interambulacral light spot is likely to be weak, thus explaining their more consistent movement towards the light spot. The interambulacral spines, on the other hand, show a greater tendency to move toward the ambulacrum, suggesting that here the ambulacral directing factor is stronger. The difference in the behaviour of the two kinds of spine in response to interambulacral stimuli would thus appear to be the consequence of differences in the relative strengths of ambulacral directing and surface factors.

However, the idea does not explain why stimulating a distant ambulacrum is more effective in directing the movement of ambulacral spines. A possible explanation will emerge later.

2. *The ambulacral directing factor*

Since the shadow reaction involves the radial nerve (Millott 1954), this structure may be the operative factor in the ambulacrum. This can be tested by removing it.

In preparations with only one radial nerve, such action abolishes the shadow response. If there are two, the removal of one does not abolish the response, but it may bring about significant changes in the direction of the first movement, as well as in other features of the response (figure 2). Thus when an ambulacrum is stimulated by a light spot on its outside surface, aboral interambulacral spines along its borders move consistently towards it (table 2) so that they converge over it (figure 1).

When the subjacent radial nerve is removed, the same stimulus causes these spines to point to the distant intact ambulacrum, so that spines bordering one side of the 'denervated' ambulacrum change the direction of their first movement, pointing to the remote ambulacrum as consistently as they did to the nearer one before the operation (tables 2 and 3, figure 1).

Ambulacral spines behave similarly, save that here their movement to the distant intact ambulacrum is markedly more consistent than it was to their own, even before its radial nerve was removed. Thus spines which moved to the centre of their own ambulacrum with an index of 0·67, after this ambulacrum had been 'denervated', moved to the neighbouring intact ambulacrum with an index of 0·94 (tables 2 and 3).

The consistency of direction attained, appears comparable with that seen when the neighbouring ambulacrum was stimulated in a preparation with both radial nerves intact (table 2). This suggests that the responses of ambulacral spines to distant stimuli involve the distant radial nerve, a deduction which is supported by comparing the responses of such spines to stimulation of a neighbouring ambulacrum, before and after removal of their own (subjacent) radial nerve. There was no significant difference, the directional index in one case being 0·94 and in the other, 0·95.

More evidence of the importance of the radial nerve in directing movement is obtained by stimulating the nerve both photically and electrically. It was stimulated by means of a light spot 0·5 to 2·5 mm wide, projected on to it in a way already described (Yoshida & Millott 1959). Interruption of the light beam elicited movements in the nearby interambulacral spines, which moved consistently towards the ambulacrum (table 4).

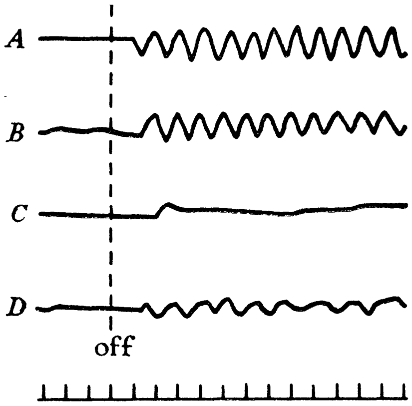

FIGURE 2. The ambulacral factor. Tracings of photographic records of the movement of one primary interambulacral spine situated alongside an ambulacrum. The preparation embodied one interambulacrum, flanked by an ambulacrum on either side. A downward movement in the records represents a movement toward the nearer ambulacrum. Time in seconds. The broken line indicates the moment at which the light stimulus was cut off. *A*, the response to a light spot projected on to the nearer ambulacrum. The spine makes its first movement towards it. *B*, the response to a similar stimulus on the remote ambulacrum, showing the change in direction of the first movement, which is now made towards the distant ambulacrum. *C*, the response to a similar stimulus on the nearer ambulacrum, but after removal of the subjacent radial nerve, showing the change in direction of the first movement which is now made towards the distant (intact) ambulacrum. *D*, the response in a subsequent experiment, following transfer of the stimulus to the intact ambulacrum.

TABLE 3. LATERAL MOVEMENTS OF PRIMARY SPINES AFTER REMOVAL OF PROXIMAL RADIAL NERVE

position of spine	position of light spot	number of trials (N)	number of movements towards distal (intact) ambulacrum (n)	directional index (n/N)
interambulacrum	proximal ('denervated') ambulacrum	31 (5)	28	0·90
interambulacrum	distal (intact) ambulacrum	30 (5)	25	0·83
ambulacrum	proximal ('denervated') ambulacrum	31 (3)	29	0·94
ambulacrum	distal (intact) ambulacrum	43 (8)	41	0·95

The nerve was stimulated electrically by glass-insulated silver–silver chloride electrodes, delivering single shocks from a standard induction coil. Judging from lack of overt effects when the electrodes were shifted slightly from the radial nerve, current spread seemed negligible. The responses of interambulacral spines to electrical stimulation of the nearest radial nerve were clearly defined, for they moved to the corresponding ambulacrum in all of the trials (table 4).

Ambulacral spines did not behave in this way; indeed, direct stimulation of the radial nerve by both means served to focus sharply differences between the two kinds of spine.

In response to photic stimulation, the spines moved to the ambulacrum with a directional index of 0·65. With electrical stimulation performance was of about the same order (0·60). In general, therefore, their directional behaviour was relatively indeterminate.

The ambulacral directing factor now appears related to the radial nerve, but it is a much more significant influence in the lateral movements of the interambulacral spines than in those of the ambulacral. Direct stimulation of the radial nerve indicates that whatever is responsible for the difference lies on the efferent side of the reflex. Nevertheless, when a distant radial nerve is involved, the ambulacral directing factor is supreme in directing both kinds of spine, and clearly overrides the effect of the surface factor.

The nature of both factors remains obscure, but some indication of the nature of the oral directing factor will follow.

TABLE 4. LATERAL MOVEMENTS OF SPINES IN RESPONSE TO DIRECT STIMULATION OF RADIAL NERVE

position of spine	kind of stimulus	number of trials (N)	number of movements towards ambulacral centre (n)	directional index (n/N)
interambulacrum	photic	70 (8)	58	0·83
interambulacrum	electrical	16 (2)	16	1·00
ambulacrum	photic	55 (4)	36	0·65
ambulacrum	electrical	30 (2)	18	0·60

3. *The oral directing factor*

The importance of the ambulacral directing factor, and in particular the power of a distant radial nerve to attract the spines within and alongside another ambulacrum, at once suggests that the spines tend to make their first movement in the direction from which nervous excitation approaches them from the operative radial nerve. This agrees with the notion that the direction from which excitation approaches a spine determines the direction of localized spine movements, but with the important reservation that in the generally accepted concept, excitation is envisaged as passing directly to the spine from the area of stimulation through the superficial nerve layer and not via the radial nerve. Photic responses necessitate revision of this idea, and the route by which excitation reaches the spine from the radial nerve assumes a primary importance.

Spine innervation

This was studied in both young and adults; whole animals or pieces were fixed in Bouin's fluid, decalcified in nitric acid–ethanol and sectioned in a plane tangential to the surface. They were stained by Mallory's triple stain, Masson's trichrome method, or by Ehrlich's haematoxylin and eosin.

Though the superficial portion of the nervous system is readily located in such material, in adults it proved difficult to follow tracts of fibres satisfactorily amid the dense felt of fibres. It was easier in young forms which, accordingly, were studied in detail.

Examination was confined to the type of spine whose movements are being studied, serial sections being followed through the bases of four primary spines (three ambulacral and one adjoining interambulacral), situated in a small sector around the aboral end of one ambulacrum.

Although very incomplete, the findings are adequate to suggest the nature of the oral factor and to explain, at least provisionally, some of the problems at hand.

In *Diadema*, as in other echinoids, a cardinal feature of nerve distribution is the emergence on the surface of branches from the radial nerve (B_1, B_2, figures 6 and 8, plate 52), by the pore-pairs which transmit the ampullary canals of the podia (C_1, C_2, figures 6 and 8). These structures define approximately the lateral margins of an ambulacrum, and therefore lie between the rows of ambulacral spines on one side and the interambulacral on the other. The emergent nerves branch in the skin (*B*, figure 9, plate 52), so that their derivatives approach the two kinds of spine from opposite directions. Excitation emanating from the subjacent radial nerve should therefore first reach the muscles on opposing sides of the two kinds of spine, the ambulacral on their interambulacral aspect, the interambulacral on their ambulacral aspect.

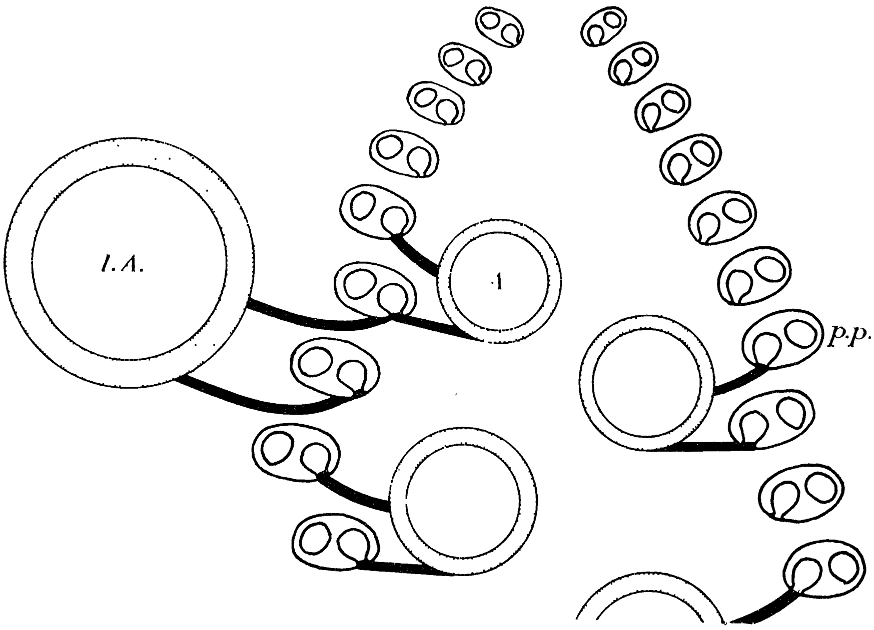

FIGURE 3. Diagram showing the innervation of primary spines, based on a reconstruction of the aboral region of an ambulacrum in a young individual (p. 445). The top of the figure is aboral. Direct nerve tracts to the spine base are shown as thick black lines. The nerve ring around the spine base is shaded; no further elements of the superficial nervous system are indicated. *A*, ambulacral spine; *I.A.*, interambulacral spine; *p.p.* pore-pair.

In the young forms at least, the spines are innervated from two pore-pairs lying alongside. The nerve to the ambulacral spines, emerging from the more aboral of the two pore-pairs, passes into the spine base approximately at the level of its horizontal diameter (i.e. 'three or nine o'clock' position) (figure 3), while the nerve more orally disposed joins the spine base near to its most oral aspect (i.e. slightly to one side of the 'six o'clock' position). The traceable direct connexions with the radial nerve are therefore predominantly on the oral side of the spine. A less pronounced, but nevertheless clear, oral bias appears in the innervation of the primary interambulacral spine. The nerve from the more aboral pore-pair (B_1, figure 6, plate 52), joins the spine base slightly oral to its horizontal diameter (i.e. at the 'three o'clock' position), while that from the more oral (B_2, figure 8, plate 52), enters at about the 'five o'clock' position (figures 3, 6 and 8).

It was difficult to trace the neuro-muscular junctions, but cells resembling neurons, with processes in intimate relation with the spine muscles on one side and extending into the nerve ring of the spine base on the other, could be seen here and there along the inner border of the nerve ring (figure 7, plate 52). In the same region are rows of nuclei, which appear to be those of similar cells, so that these could well mark the areas where neuro-muscular junctions occur.

Some of these areas occur near to the point where the nerves just described enter the nerve ring, and in ambulacral spines they are found in the short arc which defines the most oral aspect of the spine base (i.e. at the 'six o'clock' position). Therefore some nerve fibres, on entering the nerve ring, could make more or less immediate connexion with the most orally disposed spine muscles.

This may help to explain the tendency of aboral spines to point obliquely to the oral pole, for in both types of spine such an anatomical disposition could well mean that the shortest connexion with the radial nerve joins the spine base on its oral half, so that excitation might first reach neuro-muscular junctions situated preponderantly in the oral sector of the spine base.

More specifically, however, if both nerves to the spine carry motor fibres, excitation from the radial nerve would approach the spine on a broad front extending over its oro-lateral aspect, so that the spine would be subjected to lateral as well as to oral influences. But the relatively greater oral displacement of the supposedly most direct connexions of the ambulacral spines could well mean that excitation would first reach a greater proportion of orally disposed muscles in these spines than in the case of their interambulacral counterparts. There is some experimental evidence for this (p. 449). Maybe this explains the two particular differences already emphasized (pp. 440 and 442) between the behaviour of the ambulacral spines and that of the interambulacral, in response to excitation from the subjacent radial nerve, namely, the greater propensity of the ambulacral spines to point orally and, in complementary fashion, their directionally less determinate lateral movements.

Similarly, there is a difference in the disposition of the pore-pairs corresponding to the two types of primary spine. They do not form an even row on either side of the ambulacrum, but every third pore-pair is displaced toward the ambulacral centre and therefore away from the interambulacral spines and nearer the ambulacral. The displaced pair always lie alongside the most oral aspect of an ambulacral spine base. This occurs throughout most of the length of an ambulacrum and its appearance in the aboral half of a cleaned, dried test is shown in figure 10, plate 53 (facing p. 469). Though the condition was figured by Mortensen (1940) in his descriptions of *Diadema setosum* (Leske) and *D. savignyi* (Audouin) Michelin, he made no further comment.

Its significance remains uncertain, but for the matter at hand, the closer proximity of the most oral pore-pair to the spine base could imply a shorter path between the radial nerve and the most orally disposed muscles of the primary ambulacral spines. This could mean that these muscles are the first to receive excitation from the nearest radial nerve, thus increasing the oral bias.

These findings are suggestive, but they cannot be anything further until more refined techniques have made it possible to determine more adequately the location of neuromuscular junctions, the relative lengths of the nerve paths between the spines and the radial nerve, and their physiological characteristics.

It will be evident that if the oral directing factor arises in this way, it is not distinct from the ambulacral factor, for though the two have different effects, they would both depend on the precise way in which excitation from the radial nerve approaches the spine base.

The surface factor may now be considered.

4. *The surface factor*

This appears as the tendency of spines to make their first movement towards the stimulating light spot. The most demonstrative situation arises when the light spot and the operative radial nerve are disposed on opposite sides of an interambulacral spine. The first movement of the spine becomes less consistent in direction.

The effectiveness of the light spot in any position depends on the presence of a radial nerve, but since the above effect is produced in preparations with only one such nerve, it cannot be attributed to interference from an opposing reflex excited through another of these nerves. It must, therefore, be due to modification of an existing reflex through the nearest radial nerve.

The precise mechanism is obscure, but the disturbance is clearly due to attraction of the spine towards the light spot, which therefore must also exert a directional effect. It is difficult to see how, in the situation defined above, the effect could be exerted in opposition to the directional effect of a radial nerve, unless it was dependent on excitation approaching the spine base directly from the light spot.

There is clear evidence that direct pathways between the spine and sites of stimulation all round it exist in *D. antillarum* (see below), and they are known in other echinoids (Kinosita 1941). Thus, though histological preparations do not reveal discrete nerve pathways, they show a fine felt of nerves surrounding the spine base on all sides. Furthermore, when the skin is lightly touched at any point round a spine, movement occurs in the precise direction of the stimulus.

This at once suggests that the foregoing effect of light spots on spine movement is due to interaction at the spine base, between the opposing directional effects of excitation from the light spot and that from the radial nerve. Other evidence of peripheral, nervous interaction in *Diadema* has already been produced (Millott & Yoshida 1960*b*).

It is logical to ascribe to a similar cause the disturbing effects on orally directed movements of light spots projected aborally with respect to the spines (p. 440). Other possibilities exist, for efferent excitation emerging from the radial nerve by branch nerves aboral to the spine might reach it through superficial efferent nerves, and tend to direct the spines aborally. If this were so, the effective excitation would not only travel a longer route than the most direct available, but it would also be difficult to account for the oral bias of these spines, for the mechanism suggested on p. 446 would not operate unless the effective efferent excitation reached the spine via branch nerves issuing alongside.

Thus, the idea that the direction of the first movement of a spine is determined by excitation, not all of which passes through the radial nerve, would explain the somewhat erratic movements of the interambulacral spines when the light spot and ambulacrum are on opposite sides of the spine.

This brings into focus a possible explanation of the tendency of ambulacral spines to move to a light spot projected on to the centre of their own ambulacrum. In view of their innervation (p. 445), excitation from the radial nerve approaching these spines from the pore-pairs, should direct their first movements to the ambulacral margins. But here the ambulacral factor is weak (p. 442) so that the surface factor and particularly the oral directing factor arising from the marked oral bias of their nerve supply, predominate. The behaviour of the spines, though rather inconsistent, emerges as an obliquely oralward movement with a lateral component slightly biased in the direction of the ambulacral centre (p. 442 and table 2). The lateral movement could thus be the effect of the surface factor.

The possibility of interaction between surface factors was examined by projecting simultaneously two light spots equal in size and intensity on to the body surface at about equal distances on opposite sides of an interambulacral spine. In some experiments the spots were disposed longitudinally along a meridian; in others, parallel to the ambitus. In both cases the spots tended to counteract each other, so that any tendency to move towards the aboral side was checked by the oral light spot. Similarly, tendencies to move toward the interambulacral side were checked by a light spot on the adjoining ambulacrum. With surface factors thus in mutual opposition, the spines tend to move consistently in the direction from which efferent excitation approaches them.

These facts may have some bearing on the relationship already noted (Millott 1954) between the directional uniformity of a spine response and the angle subtended by the spine with respect to the stimulating light beam. When the beam is broad and directed over the spine base at a wide angle with reference to its main axis, it necessarily creates a powerful surface factor, the whole area of skin on one side of the spine base is illuminated while that on the other is in its shadow. Directional movement dominated by the surface factor is thus only to be expected.

5. *Movement toward distant ambulacra*

Experiments reported on p. 440 show that where ambulacral spines are excited by stimulation of a distant ambulacrum, their movement toward this is more frequent than to their own. This suggests that the route by which excitation approaches the spines may be different in the two cases. It is therefore pertinent to discover whether a similar improvement can be achieved by re-routing the excitation emanating from the nearest radial nerve, so that it approaches the spine from the mid-line of the ambulacrum instead of from the margin. Such a route would seem possible because the nerve ring at the spine base appears continuous with the general superficial nerve layer. Diversion might therefore be achieved by cutting the branches of the radial nerve on the same side as the spine under observation (figure 14).

When this is done (figure 15), the spine more consistently makes its first movement to the middle line of its own ambulacrum. Thus when an intact preparation is stimulated by a broad beam directed on to the ambulacrum in the area of the spine, in seventeen trials the spine moved eleven times towards the centre and six times to the margin (index = 0·65). After unilateral denervation the spine moved fourteen times to the centre and three to the margin (index = 0·82). No such improvement was observed in the lateral movements of interambulacral spines. In the light of the foregoing arguments, when lateral movements of the ambulacral spines are improved by such nerve section, oral movements should be correspondingly diminished. Shortage of living material forbade adequate verification, but in the only experiment possible, the expectation was fulfilled and the first movements of such spines were no longer orally directed after cutting these nerves.

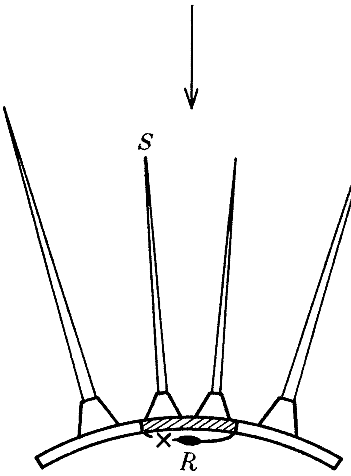

FIGURE 14

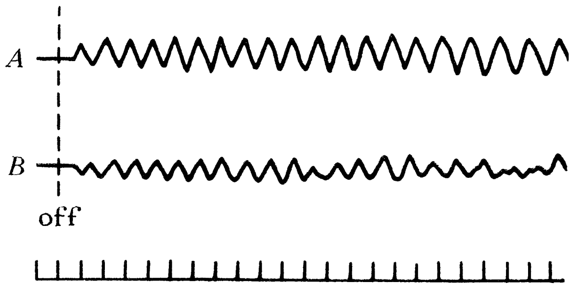

FIGURE 15

FIGURES 14 AND 15. The effect of nerve section on the direction of the first movement of ambulacral spines (p. 448).

FIGURE 14. Diagrammatic transverse section of the preparation used, showing the position (X) at which all the branches on one side of the radial nerve were cut. Ambulacral area cross-hatched. The arrow shows the direction of the light. *R*, radial nerve.

FIGURE 15. Tracings of the photographed movements of spine *S* in figure 14, made in response to shading of the ambulacral centre. The broken line shows the moment of shading. Time in seconds. *A*, the response in an intact preparation; the first movement is away from the ambulacral centre. *B*, the reversed movement which follows nerve section.

There appears therefore to be another efferent path which, in the case of the ambulacral spines at least, exerts a stronger influence on lateral movement than the more direct pathway recently discussed.

On available histological evidence, the only other possible route by which nervous excitation could reach the spine muscles is through the superficial nerve layer. In view of the extensive and diffuse character of this layer, which is continuous with the nerve ring at the spine base, it is possible for excitation from the radial nerves to reach the spines on all sides. On the basis of the argument we have developed, the effective agent determining the direction of movements of the spines will be that which reaches the spine muscles first. Disregarding possible differences in speed of conduction along the two possible routes, the effective route will be the shorter, namely, that via the branches of the radial nerve which are nearest to the spine. With the possible exception of the few interambulacral spines in the ambital region, which are situated further from the ambulacrum than those whose innervation has been studied, this must be so, for examination of sections reveals the fact that the branch nerves do not merge with the superficial nerve layer until they are almost alongside the bases of the spines of the ambulacrum (figure 9, plate 52) and those that fringe its margins. At this point, some of their fibres, in the precise positions previously described, are already entering the nerve rings of these spines.

When the route of efferent excitation is altered, by stimulating a distant ambulacrum, by cutting the branch nerves which emerge nearest to the spines under observation, or by removing the nearest radial nerve, each of which means that effective excitation must approach the spines from the undifferentiated superficial nerve layer, the peculiar conditions associated with the more direct nerve supply no longer operate. Excitation following the shortest available route now approaches the spines without the oral bias and so directs them laterally. Because the oral bias is less marked in the interambulacral spines, the improvement should be correspondingly less, and this is so.

6. *The directional mechanism*

It is now evident that the direction of the first spine movement is more than an expression of a simple reflex, being determined by two vectors, the direction in which excitation approaches the spine from the area of stimulation (surface factor) and from the radial nerve (oral and ambulacral directing factors).

The situation is more complicated when the behaviour of a row of spines extending between two radial nerves is studied. When a single light spot is projected on to the skin between different spines, so that the light and an ambulacrum are ranged at varying distances on the same or on opposite sides of the spines, the results vary in different preparations. In some, the spines directed their first movement consistently towards one particular radial nerve, whatever the position of the light spot. It is thus clear that not only may the ambulacral directing factor predominate, but also that excitation emanating from one radial nerve may override that from another. On the other hand, in some experiments the spines moved predominantly to the light spot, thus moving away from the radial nerves on both sides.

In summary, the foregoing study has tested by means of the shadow reaction, the notion that the primary spines first move in the direction from which excitation approaches them. The experiments showed the notion to be correct in principle but to involve unforeseen complications. The mechanism determining the direction of movement is not entirely superficial, but involves the radial nerve and interaction between the vectors noted above. Moreover, where there is a radial nerve on either side of the spine, the situation is complicated by the interplay between influences from these nerves.

In the course of argument, ideas about nerve pathways have emerged. Further evidence concerning them arises from a study of the latency of the shadow reaction.

CONDUCTION OF EXCITATION OVER THE SURFACE

Effective excitation can be transmitted for considerable distances over the surface. Thus in a preparation from which the oral nerve ring, periproct and all but one of the radial nerves had been removed, obliteration of a light spot on the intact ambulacrum excited movement in a spine separated from the area of stimulation by almost three interambulacra.

The excitation passes through an extensive, diffuse superficial nerve layer, in which discrete paths are mostly unrecognizable anatomically and difficult to show physiologically. Evidence concerning them may be obtained by studying the reaction time, defined as the time elapsing between the beginning of shading and the first signs of a response (Millott & Yoshida 1960*a*), when stimulus and effector are sited at varying distances from a radial nerve. The findings can usefully be correlated with evidence obtained from studying directional movement.

In these experiments, because the reaction time is a function of the stimulus intensity, lighting was standardized so as to form a spot of constant size and intensity. This was projected on to the skin for 3 min, a time known to be more than adequate to produce a maximal response with the light intensities used (Millott & Yoshida 1960*a*). Since the moment of extinction of the light was signalled on the record of spine movement, it was possible to obtain reaction times by measurement against a time trace simultaneously recorded. Where the light spot was moved care was taken to ensure, so far as possible, that the light beam was projected on to the surface of the preparation at a constant angle. When it was necessary to disturb the disposition of the light spot, in order to operate on nerves, it was replaced in its former position, but this could not be done with great accuracy. The defect was minimized by keeping all light spots on the ambulacral centre which is not only easily located, but in addition has few iridophores which could be a source of disturbance because of their light-scattering properties. Another limitation arises from the method of recording. Where first movements are small and oblique with respect to the optical axis of the camera, the onset of contraction is sometimes difficult to determine. Nevertheless, the method yielded some significant information.

Evidence from reaction times

It soon becomes evident that comparisons between the reaction times of spines have limited usefulness. Some spines differ considerably in this respect, even when they are of the same order and located in the same area. Others show prolonged shock effects in response to nerve cutting, changing their stance, or sometimes failing to react. Although many showed consistent reaction times in response to identical stimuli, this was not always so. The difficulty recalls that previously experienced when the outside surface of preparations was stimulated (Millott & Yoshida 1960*b*), and the results never attained the consistency associated with radial nerve stimulation (Millott & Yoshida 1960*a*).

In general, spines more distant from an ambulacrum proved slower to react than those sited nearby, so that a gradient exists from nearest to farthest. In preparations with two ambulacra the gradient is reversed when the light spot is shifted from one ambulacrum to the other (table 5).

Removal of one radial nerve from such preparations yields some revealing results (table 6). In the first place, if the light spot is projected on to the ambulacrum which remains intact, the operation makes no material difference to the reaction time of any of the spines. Such findings support previous indications that only one radial nerve is involved. Since this applies to spines on either side of the 'denervated' ambulacrum, they also show that transmission across an ambulacrum is over its outer surface and is not normally relayed via the underlying radial nerve.

When the light spot is projected on to the 'denervated' ambulacrum, if this bears the spine whose movements are studied, a striking difference is observed (table 6). The reaction time of the reflex, now consummated through the distal radial nerve, is increased about three times after the operation.

TABLE 5. GRADIENT IN REACTION TIMES OF FOUR PRIMARY SPINES SITUATED AT ABOUT EQUAL INTERVALS ACROSS THE INTERAMBULACRUM BETWEEN TWO ADJACENT AMBULACRA, SHOWING REVERSAL OF THE GRADIENT WHEN THE STIMULUS IS SHIFTED FROM ONE AMBULACRUM TO THE OTHER (P. 451).

Figures quoted show the mean reaction times in seconds for three observations ± S.E.

	spine I	spine II	spine III	spine IV
light spot on ambulacrum near spine I	0·870 ± 0·097	0·860 ± 0·087	0·990 ± 0·020	0·997 ± 0·049
light spot on ambulacrum near spine IV	1·107 ± 0·095	1·020 ± 0·087	0·967 ± 0·055	0·860 ± 0·087

TABLE 6. REACTION TIME OF A PRIMARY AMBULACRAL SPINE IN A PREPARATION BEARING TWO AMBULACRA, BEFORE AND AFTER REMOVAL OF THE PROXIMAL (SUBJACENT) RADIAL NERVE

The figures are mean reaction times in seconds for three observations ± S.E.

	both radial nerves intact	proximal radial nerve removed
light spot on proximal ambulacrum (i.e. that bearing spine)	0·590 ± 0·054	1·730 ± 0·075
light spot on centre of interambulacrum	1·332 ± 0·060	1·345 ± 0·077
light spot on distal ambulacrum	0·774 ± 0·019	0·936 ± 0·049

Although a difference appears, whether it is the ambulacrum bearing the observed spine that is 'denervated', or the other, it may not be attributed to differences in the speed with which reflexes are executed through the two radial nerves. Thus, when excitation is re-routed in a number of preparations, the substantial differences in reaction time observed, are always associated with the same disposition of the radial nerve with respect to the spine and light spot.

The implications of the difference will be clear from the following experiment performed on the same type of preparation. If the light spot is projected on to the ambulacrum remote from the spine (figure 16*B*, arrow 2), the afferent path to the subjacent radial nerve is short and the reaction time is relatively short. When the light spot is transferred to the ambulacrum bearing the spine and the subjacent radial nerve is removed (figure 16*B*, arrow 3), the reflex must be executed through the remaining radial nerve. Here the efferent path is the same as before, because the direction of the first movement of the spine shows that the reflex is executed through the same radial nerve, but the afferent path is much longer and the most direct route possible for the reflex would be almost double its former length. But the reaction time is much more than double, indicating that the difference is largely due to the intercalated afferent pathway.

An estimate of the transmission speed may be obtained from experiments of this kind performed on five preparations of this type (figure 16 and table 7). The transmission velocity in efferent nerves (V_e) may be calculated from the total reaction time of an ambulacral spine observed, when its own and the adjacent ambulacrum is stimulated, in accordance with the formula

$$V_e = \frac{d}{t_2 - t_1},$$

where t_1 is the reaction time of the ambulacral spine when the ambulacrum bearing it is stimulated, t_2 the reaction time when the neighbouring ambulacrum is stimulated, and d is a rough measure of the difference in length of the efferent pathways in the two cases (i.e. the width of the interambulacrum at the level of the spine). The values are set out in table 7, and the average value for V_e is 60·0 ± 3·9 mm/s, for the temperature range 21 to 23 °C.

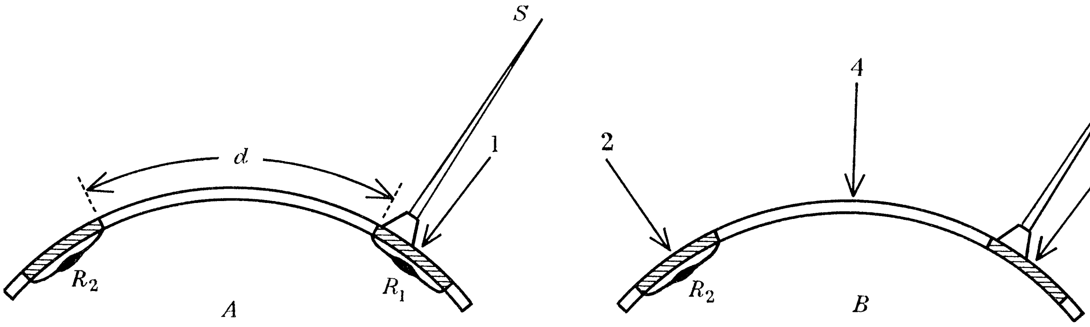

FIGURE 16. Diagram showing a transverse section of the type of preparation used to estimate transmission velocity (pp. 452 to 455 and table 7). The ambulacra are indicated by cross-hatching. *A*, the preparation with both radial nerves. *B*, the same preparation following removal of one radial nerve. The numbered arrows correspond with the positions at which light spots were projected in each type of preparation, to determine t_1, t_2, t_3 and t_4, respectively. *d*, width of interambulacrum; R_1, R_2, radial nerves; *S*, spine, the reaction time of which was ascertained.

TABLE 7. CONDUCTION VELOCITY OVER THE INTERAMBULACRAL SURFACE

For symbols see text p. 453 and figure 16.

preparation no.	t_1 (s)	t_2 (s)	t_3 (s)	d (mm)	$V_e = \frac{d}{t_2 - t_1}$ (mm/s)	$V_a = \frac{d}{t_3 - t_2}$ (mm/s)	temp. (°C)
1	0·58	0·89	1·80	20	65	22	21
2	0·83	1·23	2·14	20	50	22	23
3	0·47	0·91	1·55	20	59	31	22
4	0·55	0·88	1·90	20	54	20	22
5	0·70	0·96	2·50	20	72	17	23

The speed of afferent transmission (V_a) is given by similar data in which t_2 is the reaction time of an ambulacral spine following stimulation of the neighbouring ambulacrum, and t_3 the reaction time when its own ambulacrum is stimulated after the underlying radial nerve has been removed, in accordance with the formula

$$V_a = \frac{d}{t_3 - t_2}.$$

From the values given in table 7, the average speed for conduction over the afferent pathway is 22·4 ± 2·3 mm/s in the range 21 to 23 °C.

This can be only a rough estimate because the values used for d assume a direct nerve path across the interambulacrum. This is unlikely because of the juxtaposition of the spine bases. It is therefore too low, implying that the absolute values for transmission speed are minimal. The comparative rates are therefore more significant.

In estimating V_a another factor must be considered. The radial nerve is light sensitive (Yoshida & Millott 1959), and might therefore be stimulated directly by the light spot despite the dense pigmentation in the overlying skin. This would mean that values for t_2 and t_3 are not comparable, because the reflexes for which they are computed would have different initial mechanisms in the intact and 'denervated' preparations.

A check was made in one preparation by comparing the reaction time after stimulation of the 'denervated' ambulacrum (t_3) with that which follows stimulation of a point midway across the interambulacrum (t_4) from which the velocity (V'_a) may be calculated, using the formula

$$V'_a = \frac{d}{2(t_3 - t_4)}.$$

This gives a value of 23 mm/s at 21 °C which agrees satisfactorily with the preceding estimate.

Even when due weight is given to these considerations, it is clear that transmission of excitation over the interambulacrum can occur with speeds that, to say the least, are significantly different. The speeds may be compared with those obtained by Pople & Ewer (1954, 1955) for transmission along the circumoral and radial nerves of *Cucumaria*, the mean values for which, given in m/s, were $0{\cdot}11 \pm 0{\cdot}04$ at 20 to 23 °C and $0{\cdot}17 \pm 0{\cdot}03$ at 24 to 27 °C, respectively.

It would be premature to attribute the different speeds with which excitation is transmitted over the surface of *Diadema* entirely to differing properties of nerve, since the participation of neuro-muscular relays must be considered. Such relays are not necessarily only terminal, they could also occur at several sites in the transmission of excitation over the surface, for, though most or all but one of the spines had been removed in the preparations studied, the muscles at the spine base were left intact. Moreover, as table 5 shows, spines in successive positions across an interambulacrum may show differences in reaction time that vary by sizeable fractions of a second, which hints at a possible relay of excitation from one to the other by nerve links excited proprioceptively. The possible participation of such relays will be considered again in the succeeding section.

The situation could be very different when the circumoral nerve ring is intact, since another potentially operative nerve link would connect the ambulacra. This possibility has not yet been adequately analyzed, but it may be mentioned that a few experiments were conducted on preparations consisting of an interambulacrum flanked on either side by ambulacra, linked by an intact sector of the nerve ring. The reaction times, shown by both primary ambulacral and interambulacral spines after photic stimulation of a distant ambulacrum, were not increased when the piece of nerve ring was removed. The significance of the nerve ring in relation to photic responses remains uncertain.

Oscillation of the spines

1. *Frequency gradients*

Attention has hitherto been concentrated on the first movement of the spines, i.e. on its direction and latency. If the stimulus is photic and not too feeble, the spines continue to oscillate actively.

Responses to mechanical or electrical stimuli are different; they occur in the absence of a radial nerve and the response is not the same in character, the initial movement being followed by a very slow oscillation. The rapid beat occurs only in the presence of a radial nerve which therefore appears to be important in generating the more rapid oscillation. Millott & Yoshida (1960*a*) reported that provided corresponding phases are compared, the responses of a spine to successive identical stimuli appear remarkably similar in the amplitude of the beats and in their frequency (the number of beats per unit of time). The responses also continue for similar lengths of time. When groups of spines are considered the situation is more complex, but a degree of uniformity appears in the responses (see below).

TABLE 8. FREQUENCY OF OSCILLATION OF THREE SPINES RANGED ACROSS AN INTERAMBULACRUM IN A PREPARATION WITH TWO AMBULACRA, BEFORE AND AFTER REMOVAL OF ONE OF THE RADIAL NERVES

Average frequencies of oscillation for the initial 10 s of the responses are shown as the number of beats per second. Values lower than 0·5 beat/s are necessarily only approximations, since the oscillation becomes very irregular at low frequencies.

		spine I	spine II	spine III
both radial nerves intact	light spot on ambulacrum near spine I	1·3	1·0	1·1
	light spot on ambulacrum near spine III	0·9	1·0	1·1
radial nerve near spine I removed	light spot on ambulacrum near spine I	0·1	0·2	0·4
	light spot on ambulacrum near spine III	0·6	0·8	1·0

Because of difficulties in focusing, the movements of no more than four primary spines, situated near together and approximately in the same plane, could be photographed simultaneously. Attention was confined to groups of such spines situated aborally to the ambitus.

In response to a common stimulus, the oscillations of the spines composing such groups often show a similar correspondence in amplitude, frequency and duration. Records of their overall activities show similar 'envelopes', and appear like those of responses to a common drive. This degree of correspondence does not exist between the responses of the two categories (ambulacral and interambulacral) of primary spine, but only between members of the same category (figure 11, plate 53). Thus the ambulacral spines tend to oscillate with a higher frequency than their interambulacral counterparts.

Closer studies of the frequencies, however, reveal significant differences. The spines which oscillate with the highest frequency are almost invariably near a radial nerve, and their frequency tends to decline as their distance from the nerve increases. This may not be evident in preparations with more than one radial nerve, until one nerve is removed. The effect is recorded in table 8, compiled from preparations with two radial nerves, one of which was removed during the course of the experiment.

It will be seen that a steep gradient in frequency extends laterally from the remaining radial nerve. The gradient appears wherever the light spot is placed on the outside surface, but if it falls on the intact ambulacrum, the frequency of oscillation is much greater than when it falls elsewhere. This is particularly striking when the 'denervated' ambulacrum is shaded. The gradient observed is as clear as before and extends from the intact ambulacrum, but the highest frequency recorded (that of the spine alongside the intact radial nerve) is greatly reduced.

The situation in preparations with two radial nerves is not so clear. Often there are no signs of gradients. When they exist across the intervening interambulacrum, however, they are never so steep as when there is only one such nerve (table 8). In some cases signs of a double gradient appear, extending from each ambulacrum to the interambulacral centre, but there are never enough spines to make a convincing demonstration. It is clear, however, that one radial nerve interferes with the effect of the other, and the unconvincing pictures observed could be the result of such mutual interference, bearing in mind that the distance between the spines is small, even at the ambitus.

It is significant that all the gradients extend latitudinally. There is no evidence of gradients extending meridionally aboral to the ambitus. The gradients therefore occur where spines are situated at varying distances from ambulacra and are associated with pathways outside the radial nerve.

These observations strengthen the deduction made on p. 455 concerning the importance of the radial nerves in the generation of oscillation, and show that their effect declines with distance. This may be distinguished as the efferent decrement. The marked fall in frequency of oscillation of a particular spine, that occurs when the stimulus is shifted from a position near an intact radial nerve to a position more remote from such a nerve, despite the fact that the imparted stimuli are identical (p. 455), shows that there is also a decrement on the afferent side of the reflex.

When the frequency gradient is correlated with observations on reaction time and the direction of the first movement, significant comparisons emerge.

In a preparation with two ambulacra, the spines of one point towards the other ambulacrum when the latter is stimulated by a light spot (p. 440), which suggests that efferent excitation is approaching these spines over the surface and not through the radial nerve that lies immediately beneath them. Similarly, spines located at progressively greater distances from the stimulated ambulacrum show a corresponding increase in reaction time. The gradient in latency therefore conforms with directional movement and indicates that excitation is passing over the surface and not through the local radial nerve.

The influences that determine frequency have a different origin, or take a different route, because the spines oscillating with highest frequencies are those nearest a radial nerve, whether this be the one in the stimulated ambulacrum or not. The gradients associated with frequency and latency do not therefore conform.

The importance of the radial nerves in maintaining oscillation can be shown by subjecting them to changes in temperature.

2. *The role of the radial nerves*

(*a*) *As pace adjustors: their effect on spine oscillations excited by photic stimuli*

The radial nerves were stimulated by projecting on to them light spots which were essentially the same as those previously employed. Temperature was changed in two ways. By raising or lowering the temperature of whole preparations, using a coil carrying hot or cold water, immersed in the circulating sea water of the experimental tank. Alternatively, localized cooling was achieved by a capillary loop about 1·0 mm in diameter, placed along the internal radial structures (including the nerve) nearest to the spine observed. The aim was to cool approximately 1·0 cm of nerve. Ethanol, cooled by solid CO_2, was circulated through the loop. Different temperatures were obtained by varying the rate of circulation. To avoid chilling the surrounding tissue, sea water circulation was increased to the maximum possible, and cooling of the sea water was minimized by enclosing the cooling capillary in an air jacket, so that only the part of the loop applied to tissue was in contact with the circulating water. Temperatures were measured by thermo-couples sensitive to 0·1 °C, placed in contact with the chilled tissue. Each experiment was preceded by a check to ensure that the temperature remained constant for at least 1 min before the stimulating light was switched on. Illumination was continued for 3 min.

TABLE 9. EFFECT OF TEMPERATURE ON REACTION TIME AND FREQUENCY OF SPINE BEAT IN ONE PREPARATION (MEAN VALUE ± S.E.). SEE P. 457

temperature (°C)	reaction time (s)	frequency (beats/s)	number of trials
17	0·973 ± 0·091	0·320 ± 0·011	3
22	0·752 ± 0·062	0·608 ± 0·013	5
27	0·530 ± 0·010	0·248 ± 0·022	4

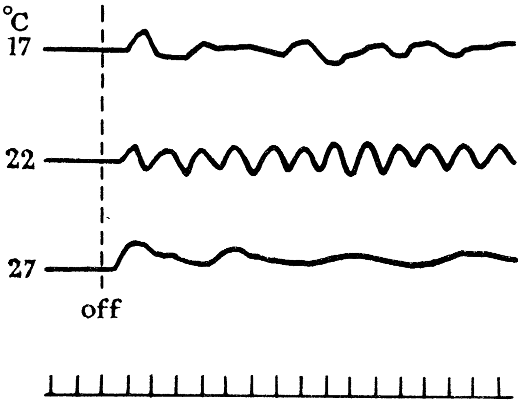

FIGURE 17. Tracings of records of the shadow response of an interambulacral spine, showing the effect of temperature on the beat (p. 457). The temperature corresponding to each record is shown alongside and the onset of shading by the broken line. Time in seconds.

The effect of temperature. Changing the temperature of the circulating sea water, and therefore that of the whole preparation, exerts a marked effect on the latency and frequency of spine oscillation (table 9). Cooling from 22 to 17 °C lowers the frequency and increases the reaction time. Warming to 27 °C again lowers the frequency but decreases the reaction time, so that frequency becomes optimal at 22 °C and the reaction time is lowest at 27 °C (the highest temperature used).

It is noteworthy that the regularity of the spine beat was also affected, varying with the frequency and becoming optimal at 22 °C (figure 17).

Changes in temperature are likely to affect the various components of the reflex, receptive, conducting and contracting, but localized cooling shows that the radial nerves are significantly and specifically affected.

The effects are shown in figure 18. The abscissae represent the temperatures recorded by the thermo-couple. It must be emphasized, however, that such temperatures were not those to which the radial nerve was subjected because of the unknown thermal conductivity of the surrounding tissue. It cannot therefore be assumed that its temperature changed to the same degree and in exactly the same way as that of the capillary loop. The graphs therefore represent only the general trend which follows change in temperature.

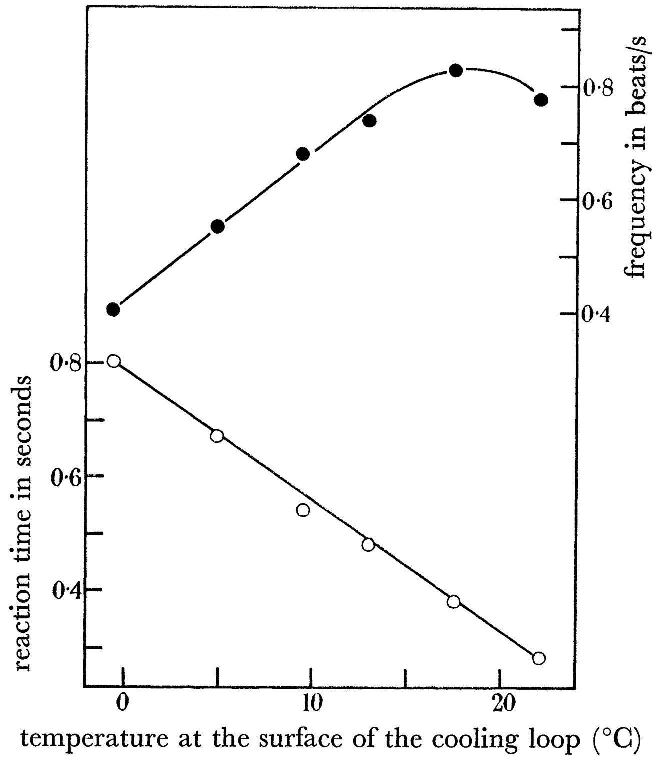

Figure 18. The effect of chilling a radial nerve (p. 458), on the reaction time (○) and frequency of oscillation (●), in the shadow reaction of a primary interambulacral spine.

Significant cooling was confined to the internal radial structures, because when another thermo-couple was placed on the outside surface, at the base of a spine immediately above the chilled section of the radial nerve, the fall in temperature recorded was not more than 0·3 °C, a change unlikely to affect seriously the neuro-muscular mechanism at the spine base or the superficial nerves.

It must be re-emphasized that when stimulated directly, the radial nerve is serving as a receptor as well as nerve centre and it is essential to know how much the receptive mechanism is affected by cooling. This becomes evident when stimulation is transferred to the outside surface of the ambulacrum corresponding to the chilled radial nerve. The effect on the frequency and regularity of the spine movements is as marked as when the radial nerve is stimulated directly.

The radial nerves are thus specifically affected in their capacity as centres for the reflex and not as receptors.

Finally, examination of the concomitant effect of temperature on latency, frequency and regularity shows, most significantly, that it is maximal at different temperatures (table 9, figure 17). The frequency and regularity of the spine beat are therefore not determined by the same mechanisms as the latency of the reflex. It may be noted that since during each experiment no light is re-admitted after the initial cut-off, shading is infinite and so the inhibitory mechanism described by Millott & Yoshida (1960*b*) does not here come into play.

The importance of the radial nerves in the generation of the spine beat is thus fully substantiated.

(*b*) *As pace makers: their effect on spontaneous oscillations*

The spine movements hitherto studied have been excited by reflex action through centres in the radial nerve. It is characteristic of such centres to show spontaneous activity and this would be particularly appropriate to those with pace-maker activity. The overt expression of such activity could appear as spontaneous and more or less simultaneous outbursts of waving in groups of spines.

It has long been known that the spines of *Diadema* show outbursts of waving (Millott 1954) and further, we now have evidence that these are unrelated to any demonstrable stimulus (see below). The extent to which such movements occur varies greatly; in freshly made preparations of the type used in this account, they may be disconcertingly prevalent, and though they usually diminish markedly within an hour, they do not disappear.

The bursts of activity may affect isolated spines or whole groups of them, so that it is not always clear from direct observation whether they are merely localized responses which, under certain circumstances, may spread, or whether they are expressions of a drive from spontaneously active nerve centres. Some spines, for example, may oscillate continuously, but show enhanced activity in concert with the periodic outbursts of waving of their neighbours.

These outbursts among groups do not appear to be due to the spread of excitation as a result of moving shadows cast on the photosensitive skin by the movements of isolated spines, for they may be seen in dim red light (see below) which is known to be ineffective in eliciting the shadow response (Yoshida & Millott 1960). If, on the other hand, they are caused by excitation leaving the radial nerve, we should expect some evidence of the gradients of activity already discovered (p. 455) and of a directional effect, so that initial movements should be towards an ambulacrum. Both of these requirements are fulfilled, but not in all cases.

Slow recordings, lasting for an hour or more, were made. Precautions were taken to avoid stimulation. Because of the photographic method of recording, it was not possible to eliminate light, but the beam used for recording movement was restricted to the spine tips, which are known to be insensitive (Millott 1954). Additional precautions were necessary because preparations increase in sensitivity in dim light, and variations such as flicker in the stray light from such beams are known occasionally to be sufficient to elicit responses. The intensity of the beam used for photographic recording was reduced to a minimum by means of neutral filters or a Wratten 25 filter which transmits only red light, cutting off completely the spectral region in which such preparations are known to be sensitive (Yoshida & Millott 1960). To avoid flicker, the lamps used were supplied by large capacity accumulators and to obviate reflexions from bubbles, aeration was suspended during recording. To cushion mechanical shocks and vibrations from the camera motor, all the main pieces of apparatus were mounted separately on pads of latex foam.

Recordings confirm that outbursts of spine waving occur at irregular intervals (figure 13, plate 53). In view of the foregoing precautions they appear to be spontaneous. As compared with responses to photic stimuli, the contractions are slower and notably less regular. It is also evident that bursts of activity occur at about the same time in several spines, though small differences in timing would not be apparent in such slow recordings.

The importance of the radial nerve is shown by the following. First, when it is removed, in most cases the co-ordinated outbursts cease; in the few cases where they persisted, synchronization was impaired. Secondly, the first of the contractions in the train of spontaneous movements is frequently directed to an ambulacrum and, further, this directional character is lost when the radial nerve is removed. Again, lateral gradients exist, extending from the radial nerve in the manner already described, so that spines nearest a radial nerve oscillate at a higher frequency, more regularly and for longer periods than those more remote.

There is thus evidence that spontaneous activity of the spines can arise in radial nerves, though it is equally clear that not all such activity arises in this way.

3. *The participation of proprioceptive mechanisms*

The importance of the radial nerves in generating the rhythm of oscillation is clearly indicated, but the question remains as to whether their effect on the main body of spines is direct, by subjecting them to a common drive, or whether it is indirect, first exciting the response of certain spines, which might then by their movement excite others. Such proprioceptive mechanisms could be important, not only as a means of relaying excitation from spine to spine, but also as a method of maintaining oscillation, once the spine has been excited in reflex fashion by the light stimulus. They could thus, by continuing to influence each other, achieve a measure of co-ordination between groups of spines.

No clear answer was obtained because it is difficult to eliminate completely possible proprioceptive mechanisms in the many minute spines interspersed among the larger ones. They are too small and too numerous to clamp and removing them is likely to inflict serious damage on possible nervous pathways in the skin.

Nevertheless, it was possible to eliminate the participation of primary spines by clamping them. Such action should affect profoundly any mechanism in these particular spines depending on movement or tension, and provided that spines in a variety of positions with respect to the light spot and ambulacrum be clamped, an effect is to be expected on the responses of some, at least, of the remaining free spines.

Primary ambulacral or interambulacral spines were immobilized in preparations essentially similar to those already used, by means of a diaphragm of coarse-meshed cloth stretched across a loop of stiff wire which could be lowered on to the tip of a spine and held firmly in a clamp. The slender spine tip with its recurved barbs thus firmly impaled, effectively resisted free angular movement at the spine base. A selection of spines was thus clamped either before the onset of contractions following photic stimulation of the skin, or for varying lengths of time after they had begun. In most cases preparations with two spines were used, one of which was clamped, and the effect of this on the other was determined by comparing the responses of the latter to a standard stimulus when its partner was clamped or free.

Such clamping did not in most cases exert any effect on the waving movements of the free spines, or indeed, on the behaviour of the clamped spine after its release, provided this was not too long delayed.

Thus, clamping a spine before stimulation and during the whole experiment exerted no significant effect on the latency, amplitude, frequency and regularity of the oscillations of the remaining free spines (figure 19).

Clamping a spine, which after stimulation had already begun to oscillate, for periods between 2 and 19 s, had no effect on the other spines.

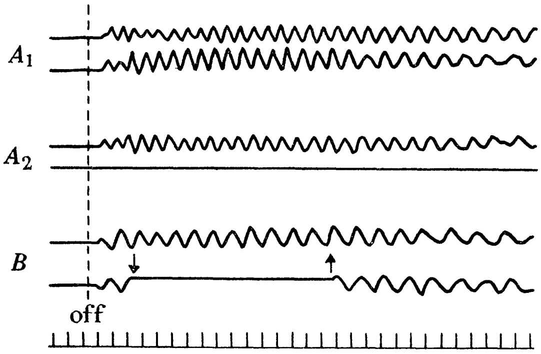

FIGURE 19. The effect of clamping a primary spine (p. 461). Tracings of the shadow responses of two adjacent ambulacral spines from three experiments. The broken line marks the onset of shading. Time in seconds. A_1, responses of the free spines. A_2, effect of prolonged clamping of one of the same spines. B, effect of briefly clamping one of a similar pair of spines, after its shadow reaction had begun. The downward arrow marks the moment of clamping, the upward, the moment of release.

The clamped spine, on release, showed variable effects. In many cases the oscillation pattern appeared essentially similar to that seen when the spine had been free; in others it was shorter in duration, more irregular, or showed beats at a lower frequency. In a few cases the spine did not resume oscillation. The last effect usually appeared in the experiments performed towards the end of a preparation's useful life and when clamping had been prolonged for more than 10 s.

In general, therefore, clamping exerts no significant effect.

Confirmatory evidence was sought by removing primary spines, together with most of their musculature, from a strip or zone between the stimulated areas and the spines under observation. The cuts were made so far as possible to leave the nerve ring round the spine base intact. Experiments were performed in preparations with only one radial nerve, so that no alternative route for efferent excitation was available. The operation made no difference to the spread of excitation over the interambulacrum or to the reaction time of the spines remaining.

In some experiments the bases (including the muscles and nerve ring) were removed from all but one of the primary spines intervening between an ambulacrum and a more distant interambulacral spine whose movements were recorded (figure 20). The one remaining intervening primary spine was clamped. Shading a small area of the ambulacrum still elicited a response in the free distant spine (figure 12, plate 53).

There is thus as yet no evidence that proprioceptive relays in the bases of the primary spines are involved in the spread and co-ordination of the shadow response among the complement of these spines.

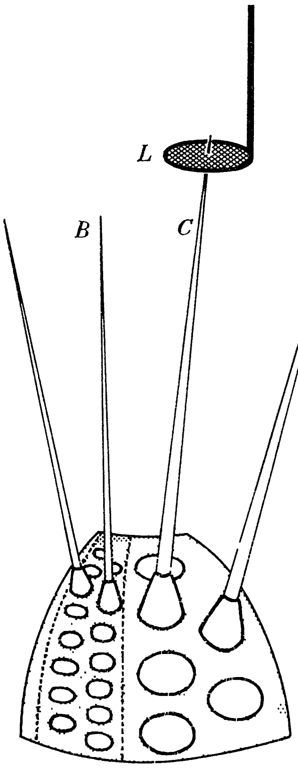

FIGURE 20. Diagram of the type of preparation used in the experiments described on p. 461. The elliptical unshaded areas represent the location of primary spines which have been removed. *A* and *B*, ambulacral spines; *C* and *D*, interambulacral spines; *L*, clamping loop. The light spot was projected between the bases of *A* and *B*.

DISCUSSION

The foregoing study of spine reactions necessitates revision of existing ideas concerning the nervous organization of echinoids in two main aspects, namely, the means whereby the spines are excited to movement and the part played by the radial nerves.

Precise knowledge of the echinoid nervous system is sadly deficient. In ordinary histological preparations, the superficial portion appears as a more or less compact but pervasive felt within the epidermis.

Von Uexküll (1900*a*), observed the spread in all directions of spine movements resulting from a local mechanical stimulus and their decline with increasing distance from the area of stimulation. He inferred that the superficial nervous system was composed of several distinct nets, through which excitation was conducted with marked decrement. More recently Kinosita (1941) has shown that conduction over the echinoid skin is not so diffuse as to suggest the existence of nerve elements formed into anastomosing networks. In wider context, Smith (1950) and Kerkut (1956), have shown that asteroids lack the degree of nervous diffuseness that characterizes coelenterates.

Critical histological and electro-physiological studies are lacking, but the foregoing study of the direction of spine movements and of conduction rates supports the idea of canalized nerve pathways. Thus, in addition to the evidence of afferent and efferent paths, the differing degree of precision with which ambulacral spines point to their own and to more distant radial nerves, indicates that excitation may approach the spine base from the same direction but in different pathways (p. 450). The differing speeds of transmission revealed in afferent and efferent pathways do not suggest the diffuse spread of excitation through an anastomosing network, but rather the differentiated tracts conceived by Kinosita (1941).

The precise means by which nervous excitation spreads from spine to spine has proved difficult to elucidate. One of the mechanisms proposed by von Uexküll (1900*a*) depends on the spreading of local reflex spine movements, initiated by the impact or pressure, of one spine on another. There is no evidence that such a mechanism plays any part in the spread of the shadow response of *Diadema*, for not only have such contacts not been observed, but the spines do not necessarily make their first movement in a direction that could lead to collision with the spines whose movements follow later.

Nevertheless, the evidence for participation of proprioceptive relays, which also seems implicit in von Uexküll's idea, is very strong as shown by Kinosita (1941) and our experiments have not precluded their participation in *Diadema*, but they cast doubt on whether such relays are always involved. Thus judging from Kinosita's tracings showing the relay of excitation from spine to spine in *Anthocidaris*, the process is much slower than the conduction of efferent excitation in *Diadema*. Accurate comparison is impossible because we cannot estimate the precise distance over which excitation was conducted in Kinosita's experiments, and *Anthocidaris* gives the impression of executing its spine movements much more slowly than *Diadema*. Similarly, the study of oscillatory gradients (p. 456) indicates decrements on both sides of the shadow reflex. Though those on the efferent side could result from proprioceptive relays, no such mechanism is involved on the afferent side, yet transmission on this side is not quicker but almost three times slower (p. 453). Unless, therefore, there is a very large discrepancy in the speed of nervous transmission on the two sides of the photic reflex, the participation of proprioceptive relays seems less likely.

More significant, however, are the disturbances in the direction of movement of the spines which result from interambulacral stimulation (p. 440). If proprioceptive relays of the type proposed by Kinosita were the sole means of conduction over the surface, it would be difficult to explain. The shadow response is clearly a reflex involving the radial nerves, so that the initial movement of the spines situated just outside the border of the ambulacrum should invariably be towards the radial nerve, and the interambulacral spines next to them should be excited by proprioceptive relays to move in the same direction. This would produce initial movements that are consistently in opposite directions, in the spines ranged between two ambulacra. They should diverge down the middle, pointing to the ambulacrum on either side. Such consistency is not observed, the spines being erratic, possibly due to interaction of the surface and ambulacral factors already postulated. Whether proprioceptive mechanisms can relay excitation over the test of *Diadema* remains an open question, but it is clear that such a mechanism is inadequate by itself to explain the efferent side of the shadow reflex.

The participation of proprioceptive mechanisms in generating spine oscillation is another matter. One of the striking features of the shadow reaction is its protracted character. This was described by von Uexküll (1897*b*, 1900*a*) and Hess (1914) in certain specialized spines of *Centrostephanus*. In *Diadema*, prolonged waving following shading has already been described as a general spine response (Millott 1954), the duration of which has been shown to be related to light intensity (Millott & Yoshida 1960*a*). This characteristic feature of photic responses has escaped the attention it deserves in general reviews of spine reactions (Hyman 1955) and merits re-emphasis, since it could well bear upon the question of proprioceptive mechanisms.

The protracted oscillation of spines could be explained in classical neuro-muscular terms in several ways. In the first place, the neuro-muscular mechanism at each spine base might simply be 'triggered' by excitation from the radial nerve. Such an idea could explain the gradient in latency observed in spines situated at increasing distances from the radial nerve, but it would not explain the observed gradients in frequency. Alternatively, contraction could be repetitively excited by proprioceptive means, so that an initial reflex movement excited by light, might in turn excite protracted contractions, not only in the spine in question, but also in neighbouring spines by virtue of the 'reflex chains' envisaged by von Uexküll (1900*a*), Holmes (1912) and Kinosita (1941). The importance of tensions developed in the spine muscles has been realized since the experiments of von Uexküll (1900*a*). Such tensions are an integral part of his scheme for explaining how mechanical pressures on a spine excite, through local reflexes, not only its own repetitive movements, but also those of the surrounding spines, by virtue of nerve connexions between them. Although there is no evidence that mechanical contact between spines is important in exciting the repetitive movements of the shadow reaction (p. 463), any shift of the long, heavy, aboral spines from their position of equilibrium is bound to alter the pattern of tensions in the musculature at the spine base.

At first it would appear possible to explain the observed frequency gradients (p. 455) in a somewhat similar way, for the oscillation of each spine could be governed by a self-exciting circuit between its muscles and their proprioceptors and centres in the radial nerve, the graded frequency of oscillation being a result of the graded lengths of the intervening pathways. The decrements could result from synapses. Such an explanation, resting on the existence of synaptic paths of increasing length, implies that latency and frequency depend on the same factors and should therefore vary together. In fact, they do not, for they have different temperature optima (p. 459), and further, as already shown (Millott & Yoshida 1960*a*), they alter in an ageing preparation at different rates. Again the experiments reported on pp. 460 to 462, though inconclusive, lend no support to the idea.

It may be noted here, that although the spines have been described as 'oscillating', their movement is often clearly two-dimensional, so that the spine tip describes what appears to be an ellipse. Von Uexküll (1897*b*) describes the spines of other echinoids as circling after stimulation of the skin. Muscular co-ordination therefore involves more than reciprocating neuro-muscular elements on opposite sides of the spine base. Complex interaction of tonic and phasic spine muscles is implied, though the nature of the interaction is unknown.

The effect of chilling the radial nerves shows that they have a significance in the shadow response over and above that already revealed, as reflex centres (Millott 1954), receptors (Yoshida & Millott 1959) and sites of interaction between excitation and inhibition

(Millott & Yoshida 1960*b*). The simplest explanation of their thermo-sensitivity would be that they cause the spines to oscillate with a frequency and regularity that is the overt image of discharges from their own thermo-sensitive association or motor neurons, the gradient in frequency of spine oscillation being an expression of the effect of synapses in the efferent tracts. A high level of uniformity in the gradients is not implicit in the idea, for spines could vary in their capacity to respond to the common efferent drive, which would be expected to change with the magnitude of the stimulus in the manner observed. However, the radial nerves might merely 'condition' spine reactivity in a decremental way. If this were so, again latency and frequency of the spines should be affected together. Other explanations are possible, though they appear unduly contrived, so that the simpler alternative is preferable. In this respect the 'drive' from the radial nerve centres would make them 'pace-adjustors' rather than 'pace-makers', in the sense that they do not generate rhythm spontaneously, the extent of their activity being determined by the parameters of lighting (Millott & Yoshida 1960*a*).

Nevertheless, there is evidence of pace-maker activity, so that the radial nerves can be seen not only as simple centres providing the initial excitation to the spine muscles, and governing the response that follows, but also as centres from which rhythms arise, apparently spontaneously. The generation of rhythm is worthy of emphasis as a newly revealed attribute of the radial nerves, and is in keeping with their function as centres of interaction in the shadow response (Millott & Yoshida 1960*b*). Both activities are appropriate to central nervous systems.* The essentially 'central nervous' character of the radial nerves has received insufficient emphasis in the past, possibly owing to pre-occupation with the idea that the circum-oesophageal nerve ring is the main centre of control.

Thus, although admitting their importance as reflex centres of podial responses, von Uexküll (1897*b*, 1900*a*), accords to the radial nerves relatively subsidiary roles in relation to spine movements, such as linking sectors of the superficial nervous system and influencing the stance and reactivity of the spines.

The direct influence of the radial nerve has been shown not to be limited to its own ambulacrum and the adjoining interambulacra, as was claimed by von Uexküll (1900*a*) who studied the effect of stimulating the nerve by nicotine, but extends at least half-way round the test, a finding reminiscent of that reported by Kinosita (1941). This means not only that a distant radial nerve may serve as the reflex centre for photic responses in areas of the skin which have had their local radial nerve removed, but also that spines may sometimes receive excitation simultaneously from more than one radial nerve. A possible manifestation of this is the indication of bilateral gradients of activity (p. 456). Co-ordination of spine movement may therefore be a much more complex affair than has yet been revealed, especially as interaction between radii in most of our preparations may have been curtailed drastically by removal of the nerve ring (p. 454).

Movements directed toward the area of stimulation have been described in other urchins. The directional response following mechanical stimuli has been explained by the

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* Since going to Press, we have seen a report by Kennedy (1963), based on electro-physiological evidence, of spontaneous discharges in another photosensitive central nervous system, namely that of *Procambarus*. Some of these discharges appear to arise in centrally situated photoreceptor neurons and the discharge pattern is modified by presynaptic sensory activity as well as by direct photic stimulation.

relay of excitation from spine to spine (Kinosita 1941), or by assuming that the spines point in the direction of approaching excitation (von Uexküll 1900*a*). Von Uexküll, believing that excitation could enter the spine base on all its aspects from the surrounding plexiform nervous system, explained the directional movements by assuming the existence of a marked decrement. The most effective excitation would therefore enter the spine base by the most direct route from the site of stimulation.

Movements directed toward shaded areas have been described in *Centrostephanus* (von Uexküll 1897*a*) and *Diadema* (von Uexküll 1900*b*; Millott 1954), but in *Diadema antillarum* at least, these movements cannot be explained on such a simple basis. They have been shown to depend on the balance between two vectors (p. 450). This is the outcome of interacting events in two sections of the nervous system, the superficial nerve layer and the radial nerves. The disposition of the stimulus and its effect in relation to that of the radial nerve (p. 447), as well as anatomical considerations, suggest the nerve ring around the spine base as the most likely site of interaction. The mechanism is therefore essentially different from that of reflexes initiated by non-photic stimuli, which can be consummated entirely in the superficial nerve layer. Von Uexküll's contention that the responses to increases in light intensity do not involve the radial nerves has been shown to be wrong (Millott & Yoshida 1959).

The precise form of the reaction as seen in isolated pieces has already been shown to depend on the spatial and temporal pattern of stimulation (Millott & Yoshida 1960*b*). The present work elaborates the spatial factor, for according to the position of the shadow, poisonous aboral spines execute their first movement in a direction determined by relative positions of shadow, ambulacrum and oral 'hemisphere'. The automatic outcome will be the protection of these areas, but there is no suggestion of the spines 'freezing' in this position as has been stated for the responses to prolonged or intermittent mechanical stimuli (von Uexküll 1900*a*), or for the so-called 'defence reactions' (Hyman 1955). On the contrary, the spines sweep repeatedly over the stimulated area. The selective advantages of rapidly guarding and sweeping over the threatened areas of the skin surface, particularly that of the most vulnerable parts of the body, the ambulacra, are tangible, but the oral spine movements appear inexplicable on this basis. Again, it may be noted that since the oral spines, which are normally applied to the substratum, do not show a response to shading, the interpretation of their supposed responses as effecting directed movements of the whole animal (Carthy 1958) is clearly erroneous.

The temporal factor requires much further analysis. Hitherto it has been disregarded except in so far as it is implicit in the assumptions that the effective excitation is that which follows the shortest path and is therefore the first to reach the spine. But interaction enhances its importance, since spines are situated at varying distances from the light stimulus and the radial nerve, so that excitation from each may reach the spine at correspondingly different times. This may to some extent account for the variable behaviour of the interambulacral spines reported on p. 450.

Although incomplete, the foregoing study has shown that existing concepts of echinoid nervous organization are not only in some respects erroneous, but also need a shift of emphasis. Following von Uexküll (1897*b*), too great an emphasis has been placed on the independence of organs such as spines in conformity with his concept of a 'Reflexrepublik'.

In wider context, more recent studies by Smith (1945, 1950) on asteroids, and by Pople & Ewer (1955, 1958) on a holothurian, have already necessitated a departure from this idea, so as to lay greater emphasis on co-ordination and integration. Smith (1945), while urging integration and marked co-ordination in the case of podia, continued to envisage independent action in spines and pedicellariae. The present work therefore signals further departure in respect of the shadow reaction of the spines.

The emerging concepts differ in several respects, but they are all incomplete and the outcome of studies on different effectors in members of different classes of echinoderms. They are therefore by no means mutually exclusive. Detailed comparisons would be premature and out of place here, but it may be mentioned that Smith lays emphasis on central nervous properties, while Pople & Ewer emphasize control by scattered motor complexes, significantly involving balance between excitation and inhibition, as well as rhythmic activity with an element of spontaneity. In a broad sense, much that has been said here and previously (Millott & Yoshida 1960 *a*, *b*) recalls their findings. In *Diadema* spine movement shows little that can be called haphazard; on the contrary, the emerging pattern is one of greater complexity and refinement, dependent in part on interacting nerve centres in both radial nerve and at the periphery (Millott & Yoshida 1960 *b*; Yoshida 1963).

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DESCRIPTION OF PLATE 52

FIGURE 4. Living *Diadema antillarum* photographed from the side, showing the body form, the disposition of some of the spines (*I.s.*) whose movements were studied and some of the anatomical landmarks referred to in the text.

FIGURE 5. A portion of the living body surface, showing the disposition of the spines whose movements have been studied. The course of an ambulacrum (*A*) has been made conspicuous by removing all but two (*A.s.*) of the primary ambulacral spines so that the exposed test shows white against the dark skin. The broken white lines around the bases of the primary interambulacral spines (*I.s.*) are due to iridophores.

FIGURE 6. Portion of a section of a very young *Diadema*, cut in a plane parallel to the ambitus and passing through the base of a primary interambulacral spine (see p. 446); showing part of its nerve supply from a branch (B_1) of the radial nerve, entering the nerve ring (*R*) around the spine base at the 'three o'clock' position. Fixed, Bouin. Stained, Ehrlich's haematoxylin and eosin.

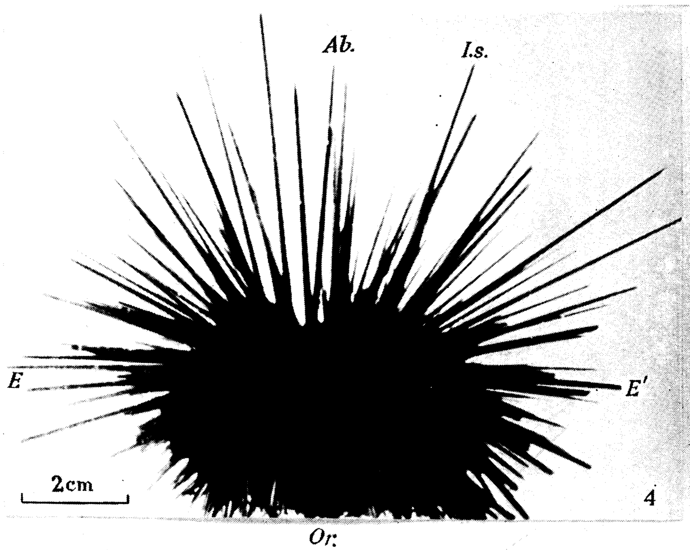
FIGURE 7. A portion of the nerve ring (*R*) around the base of a spine and the underlying spine muscles (*M*), of a very young *Diadema*, showing a motor neuron (*N*) from which pass fine processes entering into intimate relation with the spine muscles (see p. 446). The course of the processes is traced by a dotted line drawn alongside. Fixed, Bouin. Stained, Ehrlich's haematoxylin and eosin.

FIGURE 8. A part of the same series of sections as that from which figure 6 was taken, passing through the same spine, but in a plane nearer the oral pole; showing the nerves from a more orally disposed branch (B_2) of the radial nerve, leaving by the pore-pair C_2, to enter the nerve ring (*R*) around the spine base, at the 'five o'clock' position. Preparation as for figure 6.

FIGURE 9. Portion of a section passing vertically through the nerve ring (*R*) around the base of a primary ambulacral spine, showing the emergence at the surface of a branch (*B*) of the radial nerve, close to the base (*S*) of a primary ambulacral spine (see p. 450). Fixed, Bouin. Stained, Masson.

Additional lettering.

Ab., aboral aspect
Ap., aboral pole
E, *E′*, points which mark the approximate level of the ambitus
Or., oral aspect
O.s., oral spines
P, podium



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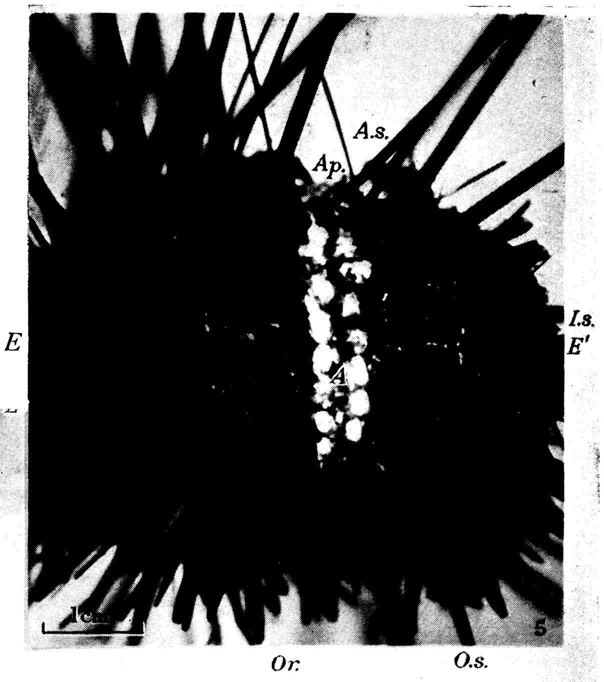

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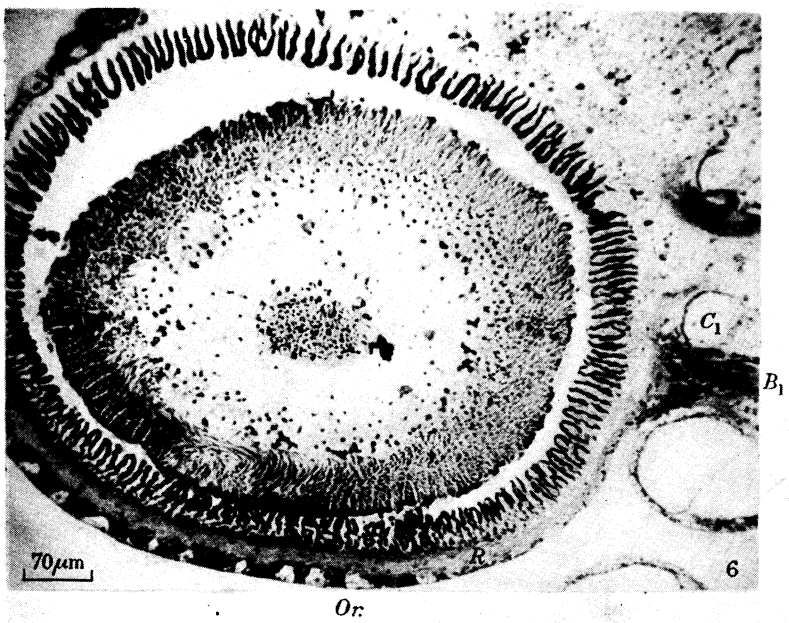

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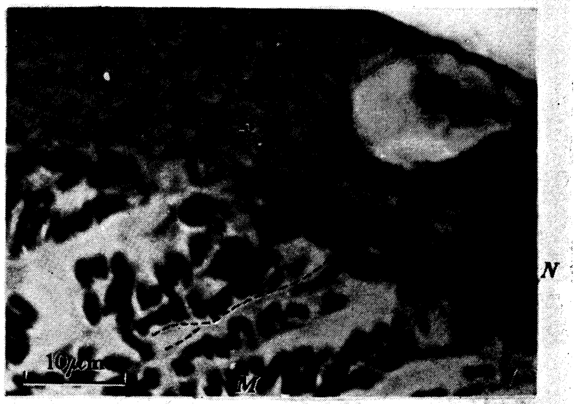

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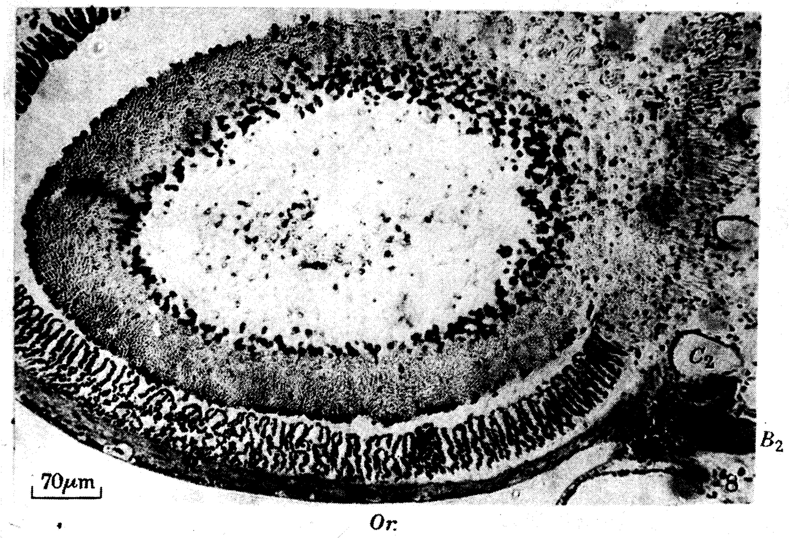

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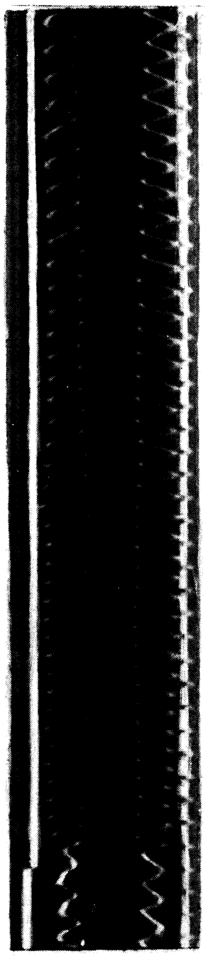
FIGURES 4 TO 9

(*Facing p.* 468)



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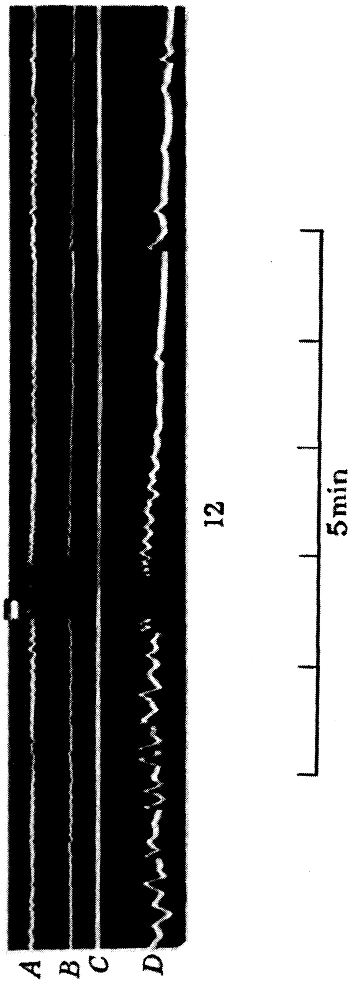

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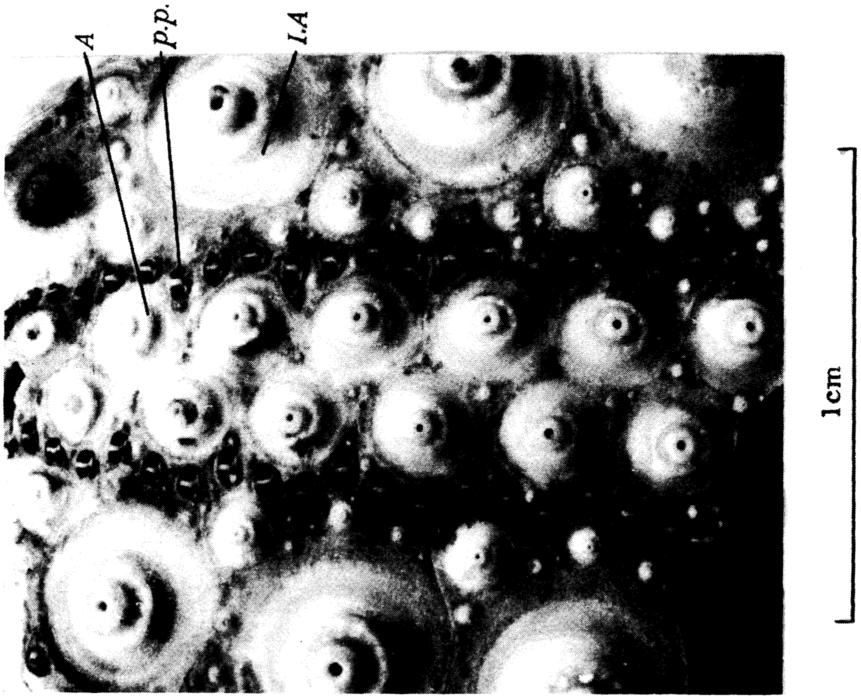

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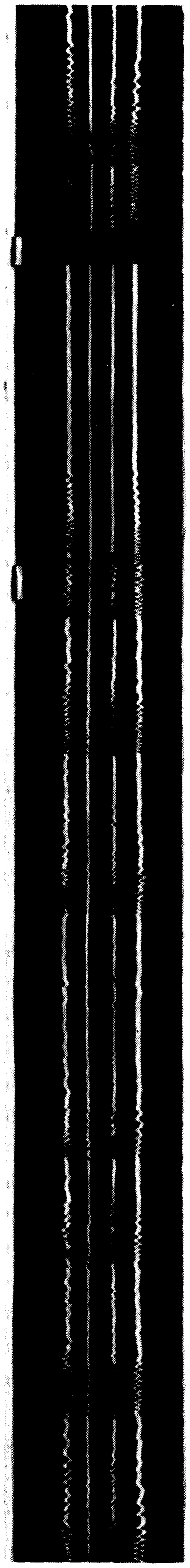

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FIGURES 10 TO 13

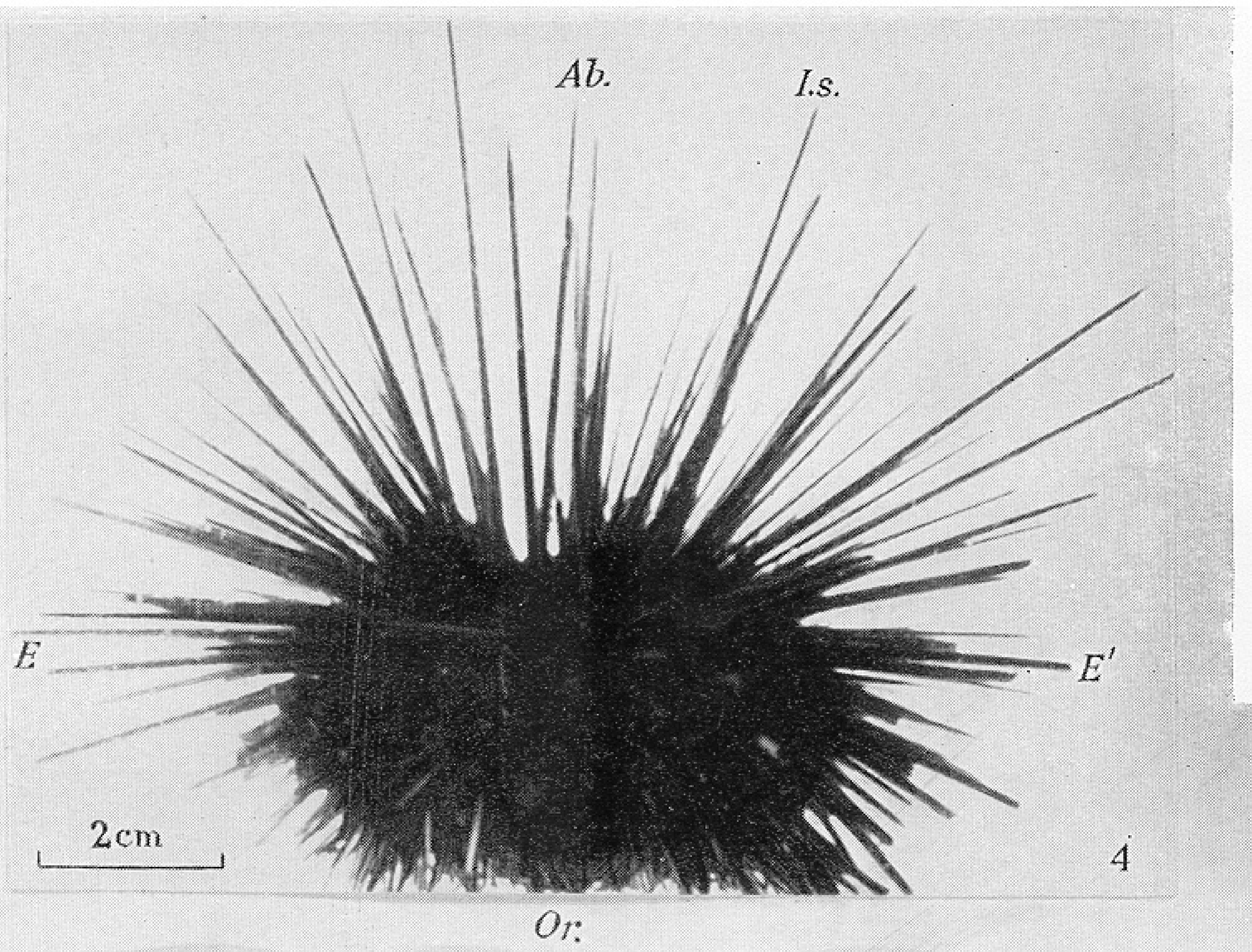
Description of plate 53

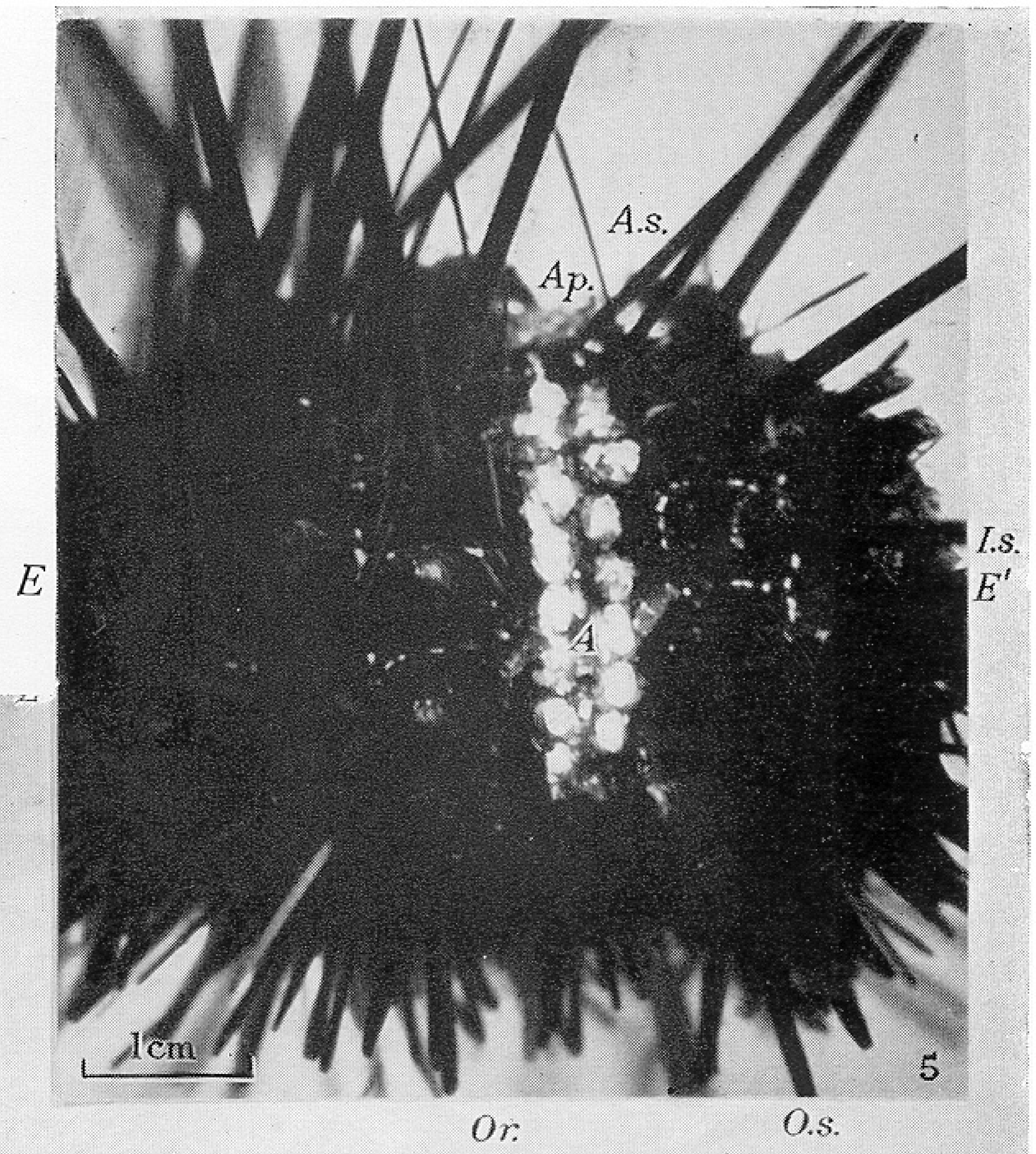
Figure 10. Photograph of a portion of a cleaned, dried test of *Diadema antillarum*, showing the disposition of the pore-pairs in the aboral region of an ambulacrum (p. 446). The most aboral region is at the top of the photograph. *A* and *I.A*, bases of ambulacral and interambulacral spines respectively; *p.p.*, pore-pair displaced toward the ambulacral centre.

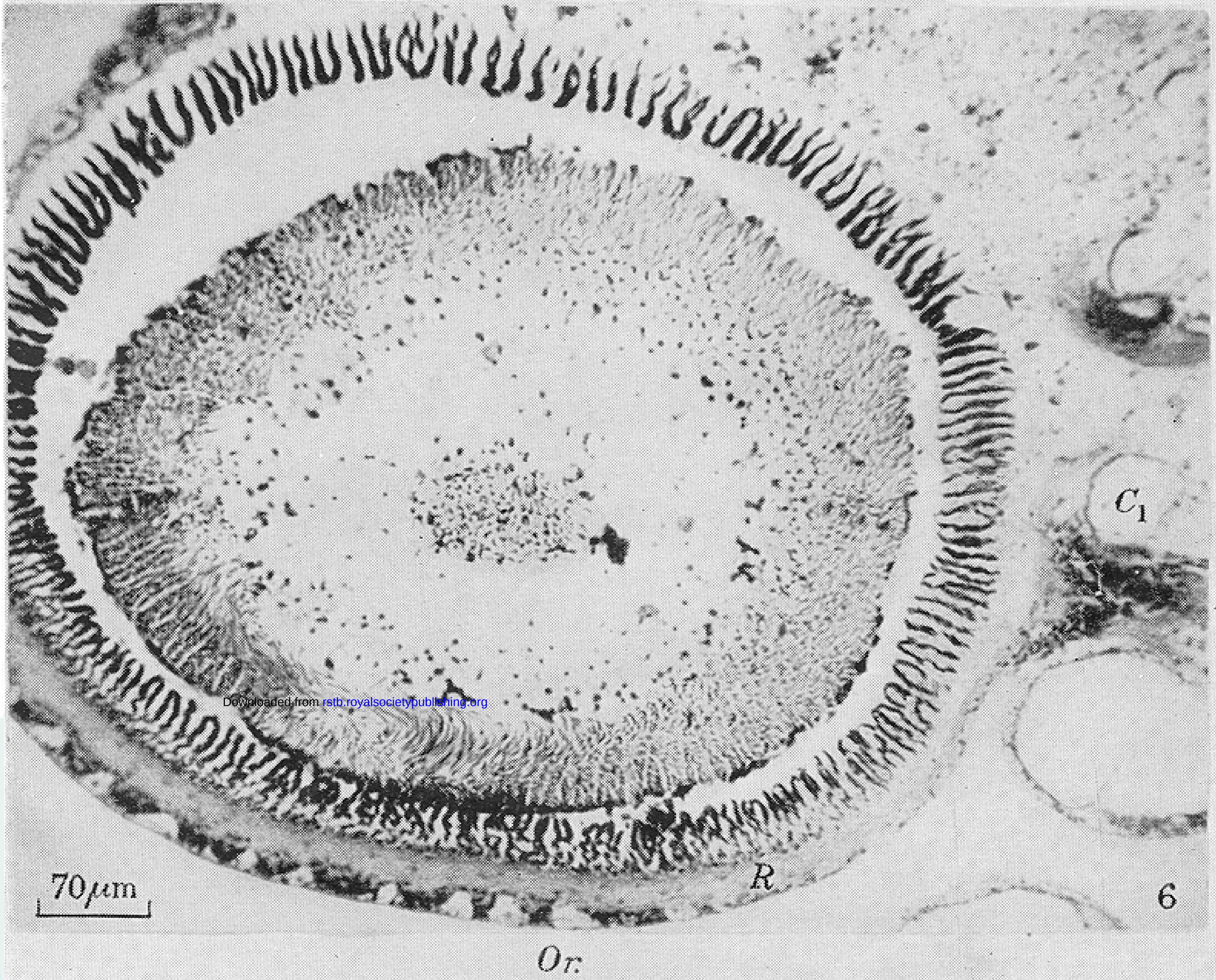
Figure 11. Record of the movements of two adjacent ambulacral spines showing the similarity of their responses to shading of their own ambulacrum (p. 455). The onset of shading is shown by the trace of the signal marker above the records. The movements before shading, which were spontaneous, also show some correspondence in rhythm. The lower trace marks the time in seconds.

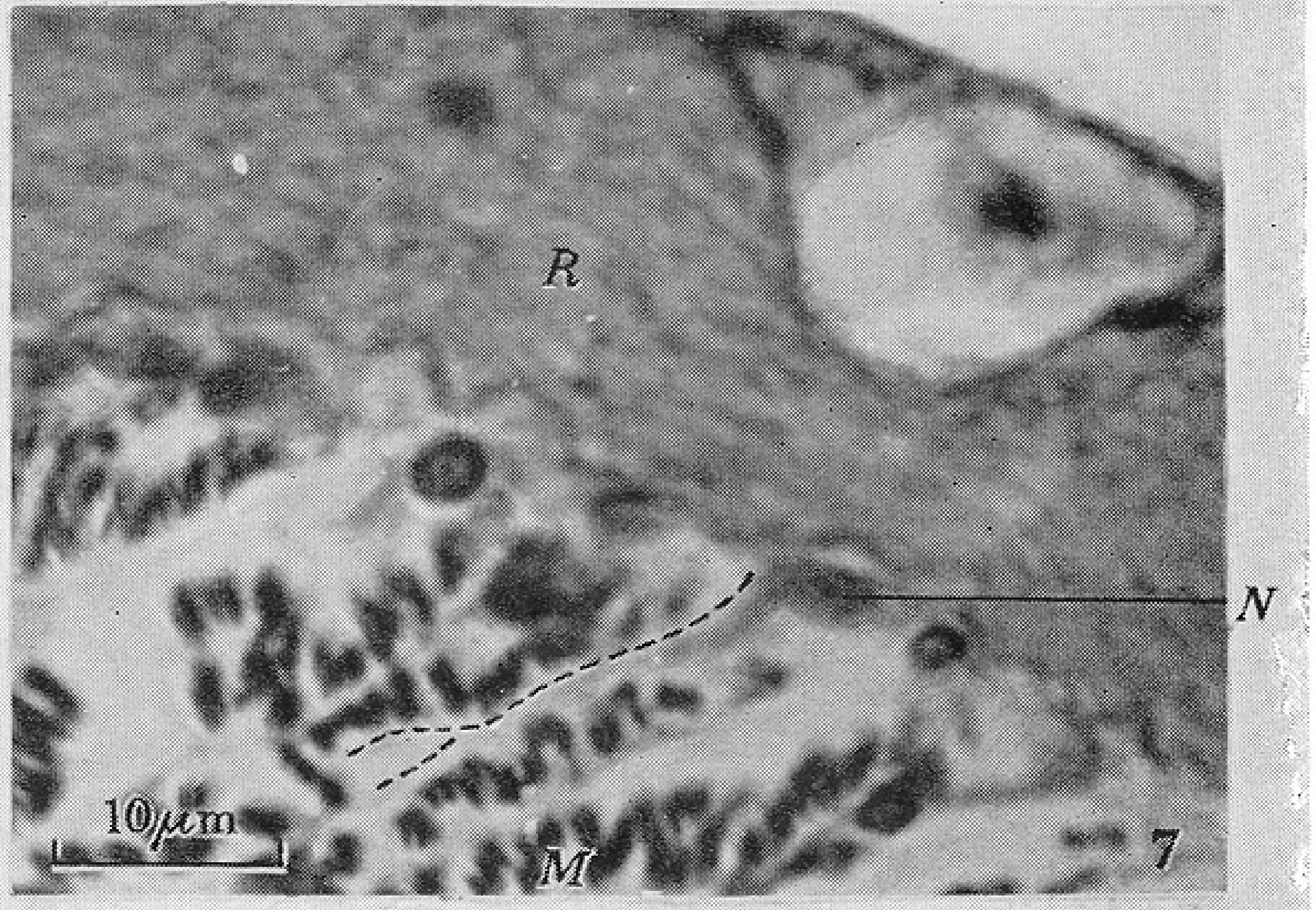
Figure 12. Slow recording, showing the behaviour following shading of the spines described on p. 461 and illustrated in figure 20. The onset of shading is marked by the downward movement of the signal. The records are marked by letters which correspond with those of figure 20, showing the spines from which they were taken. Note the spread of excitation beyond the clamped spine *C*. Speed of recording shown by the scale alongside.

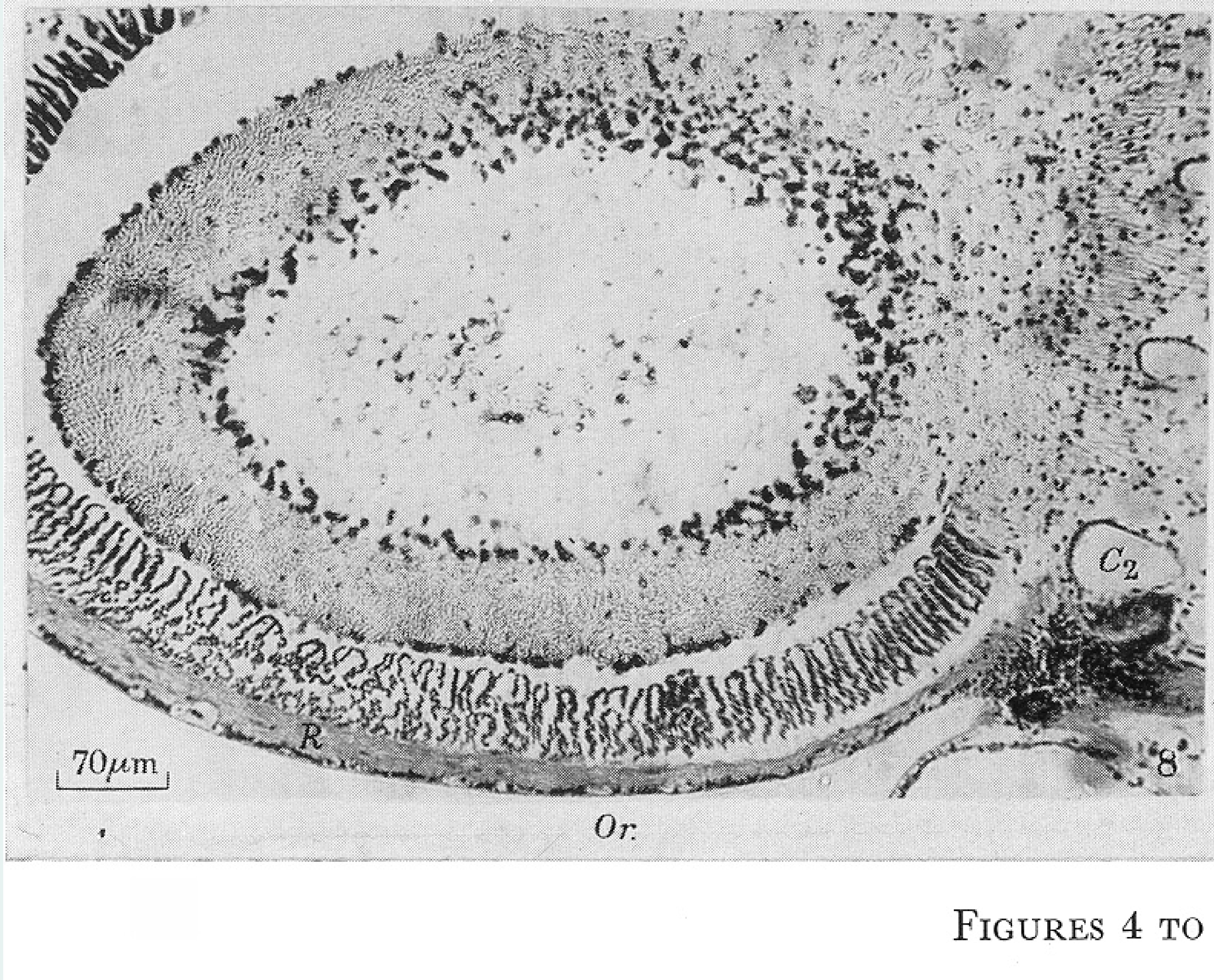
Figure 13. Slow recording showing outbursts of spontaneous movements in two ambulacral (middle records) and two interambulacral (upper and lower records) spines (p. 460). The preparation was illuminated only between the two signals. Speed of recording as in figure 12.



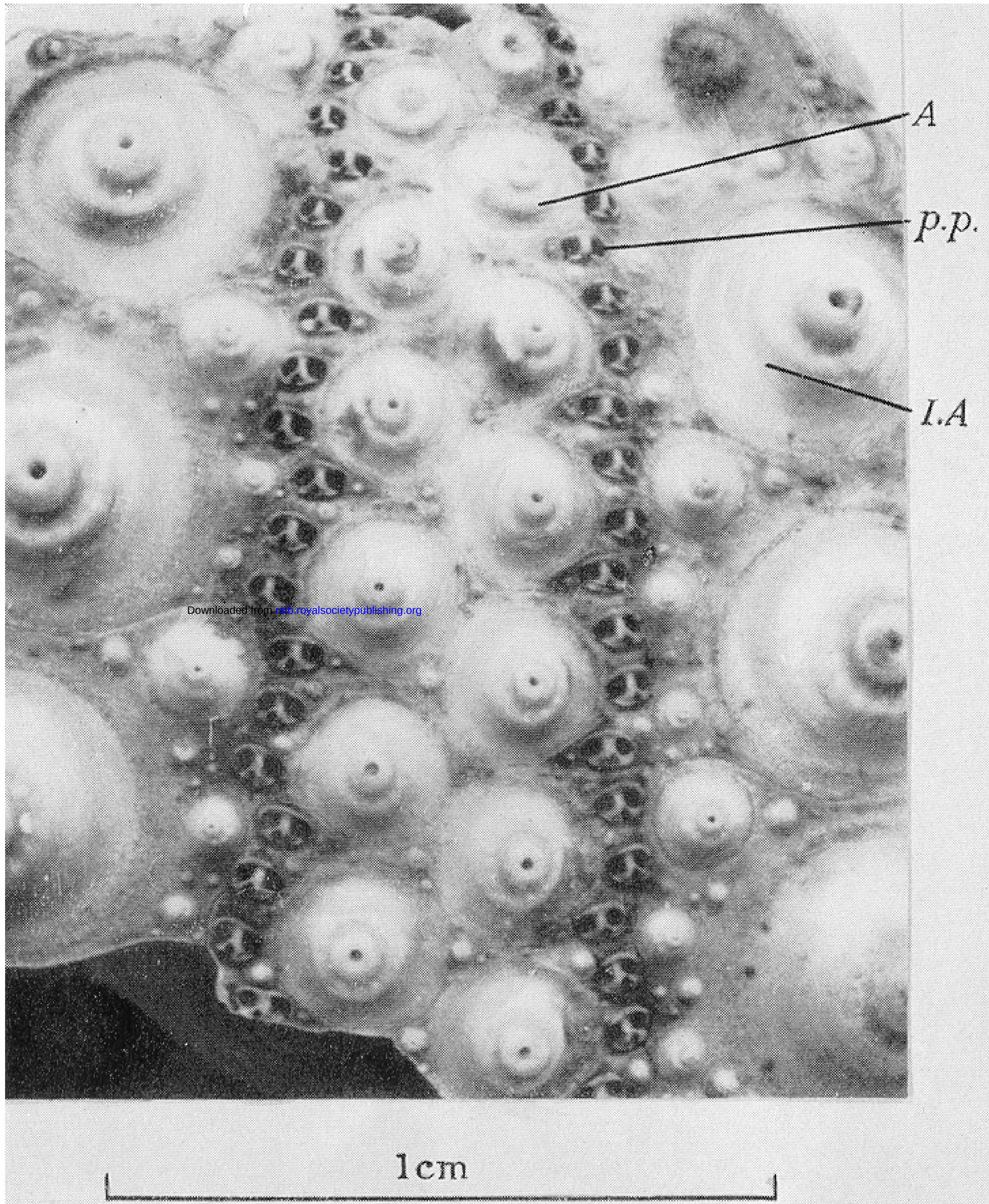








Figures 4 to 9



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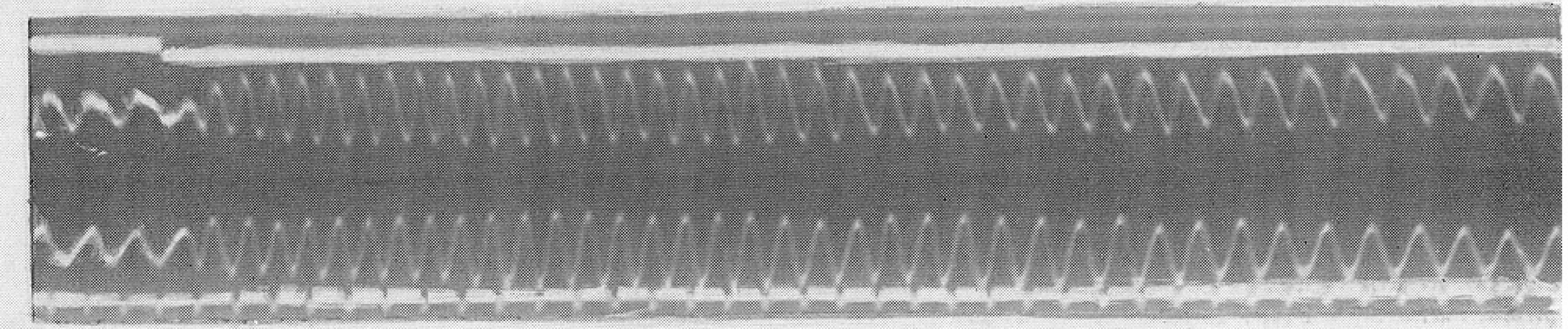

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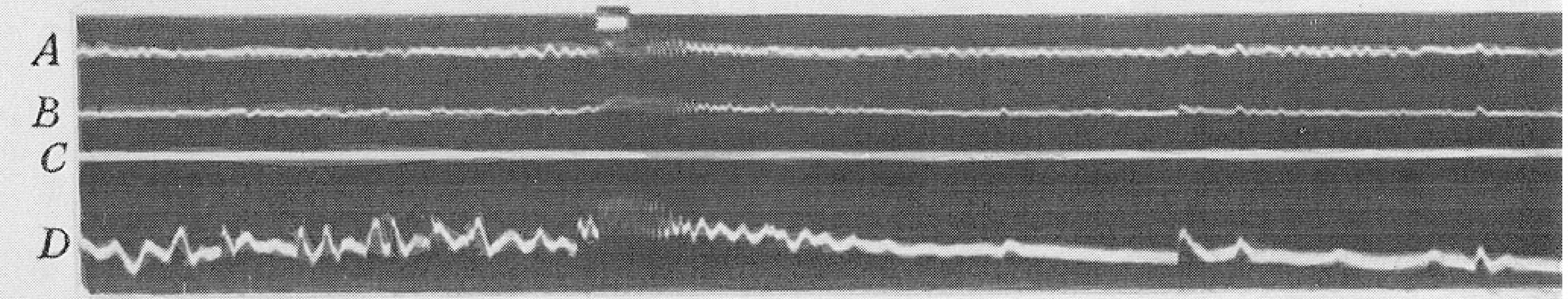

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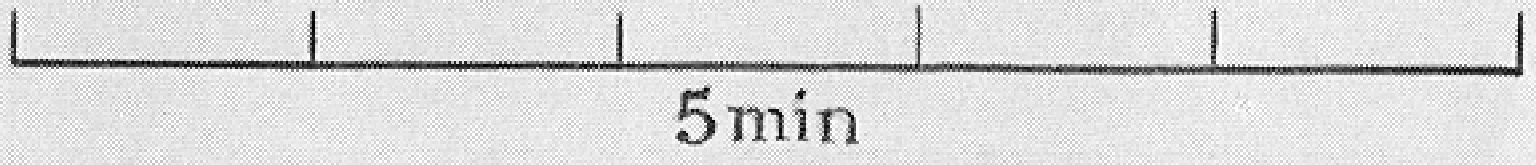

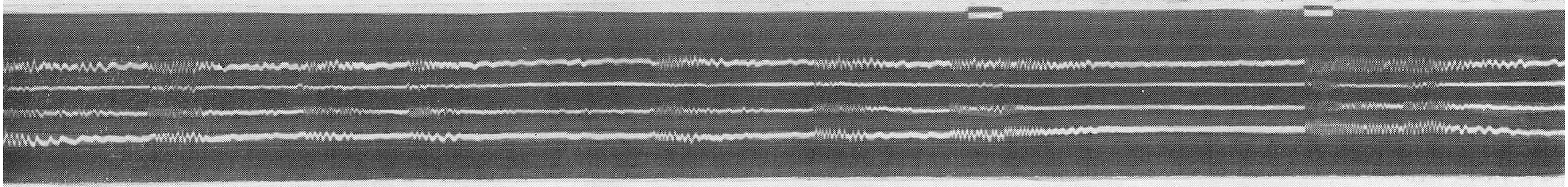

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FIGURES 10 TO 13