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STUDIES ON THE MOTILITY OF THE HELIOZOA

I. THE LOCOMOTION OF *ACTINOSPHAERIUM EICHHORNI* AND *ACTINOPHRYS* SP.

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SUMMARY

Analysis of ciné records indicates that the locomotion of *Actinosphaerium eichhorni* and *Actinophrys* sp. includes a definite rolling motion, in addition to evident horizontal and vertical displacements. Such movements could be correlated with significant changes in the lengths of supportive axopods, but not with axopodial rowing or sliding movements. The data also do not support a model of locomotion based simply on those systematic shifts in the cell's centre of gravity that would be caused by sequential collapse of supportive axopods. Although active bending of attached axopods cannot be discounted, locomotion would seem to result from forces generated between the cytosome and substratum by attached axopods undergoing changes in length. The observations suggest, moreover, that axopodial retraction is more important than elongation in the generation of motive force.

It is proposed that the relative magnitude of each locomotory component is determined by the dimensional parameters of the particular species. As a consequence, changes in axopodial length can account for both the 'rolling' and 'gliding' behaviour reported in the literature.

INTRODUCTION

The sun animalcules, or Heliozoa as Haeckel (1866) named the group, are sarcodines with spherical cytosomes and long, relatively thin and stable pseudopods (Figs. 4, 5). The heliozoan pseudopod, or axopod, has been of particular interest, since in some species it may reach a length of 500 μ (Barrett, 1958). This highly attenuated structure consists of an axial core, or axoneme, and surrounding cytoplasm that is continuous with the cytoplasm of the cytosome (Roskin, 1925; Rumjantzew & Wermel, 1925). The axoneme exhibits a striking form birefringence (Mackinnon, 1909), and penetrates deep within the cytosome. More recently, electron-microscopic studies have shown that this skeletal rod consists of longitudinally oriented microtubules; these are organized into two interlocking sheets coiled around a central axis (Kitching, 1964; Tilney & Porter, 1966). Correlated physiological and ultrastructural studies on the lability of these microtubles (Tilney, 1965; Tilney, Hiramoto & Marsland, 1966) have confirmed earlier suggestions that the axoneme is responsible for axopodial stability (Schmidt, 1944).

With respect to motility, the axopod has been implicated in locomotion and feeding,

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and contains numerous moving inclusions (see Penard, 1904, among others). Yet less seems to be known precisely about axopodial motility than is known about the pseudopodial motility of other sarcodines. For instance, the behaviour of supportive axopods has not been adequately characterized (Kuhl, 1951), and several different types of locomotory mechanisms have been suggested for these axopods (see Tilney & Porter, 1966). Precise characterization of the various kinds of intracellular motility has not been made, and in general, our knowledge of the morphology of preserved material greatly exceeds our understanding of its functional organization. The role proposed for microtubules in cellular support and motility (Slautterback, 1963; Ledbetter & Porter, 1963) warrants a more thorough study of the motility of the heliozoan axopod.

In the present paper, the locomotion of two common heliozoans, *Actinosphaerium eichhorni* and *Actinophrys* sp., has been re-examined, and a functional role for certain supportive axopods has been suggested. Other papers will deal with other features of heliozoan motility, especially with particle movements within the axopods and the cortical surface layer, and with correlated light- and electron-microscopical observations of structures associated with this motility.

MATERIALS AND METHODS

Actinosphaerium eichhorni was collected during the spring and summer of 1965 from streams at Bear Mountain, New York, and from Collier's Mill and Batsto, New Jersey. Species identification was based mainly on Leidy's descriptions (1879); general morphological features are indicated in Fig. 4A. This form was grown routinely in small samples of its native water or in medium supplemented with boiled wheat grains and an inoculum of mixed ciliates and flagellates (mainly *Paramecium*, *Tetrahymena* and *Chilomonas*) and small rotifers (*Brachionus*). In the latter instance, Marshall's medium was used routinely: $5{\cdot}0 \times 10^{-5}$M $MgSO_4$, $5{\cdot}0 \times 10^{-4}$M $CaCl_2$, $1{\cdot}47 \times 10^{-4}$M K_2HPO_4, $1{\cdot}1 \times 10^{-4}$M KH_2PO_4; made up in demineralized water (personal communication to Dr R. D. Allen). A typical culture maintained at 20 °C in a 14:10 light:dark cycle supported a dense population of food organisms for a month. The doubling time of *A. eichhorni* under such conditions was 5–10 days. Subcultures were initiated every 2–3 weeks, and only specimens from cultures 1–2 weeks old were used for microscopic examination. Except for the medium and the light cycle, the culture technique did not differ greatly from the ones employed by Lehrer (1950) and Nozawa (1938) in their growth studies on this genus.

A second form, thought to be a species of *Actinophrys* (Fig. 5) was found routinely in samples of *Sphagnum* collected from a cedar swamp near Whiting, New Jersey. To date, attempts to cultivate this organism under more controlled conditions have not been successful. However, specimens thrive and apparently multiply in 'micro-swamps'—small *Sphagnum* samples covered with 1 in. of natural swamp water (especially rich in pennulate diatoms) and contained within closed, deep plastic dishes. In all other respects, maintenance was as outlined above.

Specimens were mounted for microscopic examination at high magnification in

the culture medium between a clean slide and coverslip. The entire preparation, including an air space near the edge, was then sealed with a 1:1:1 mixture of Vaseline, lanolin, and paraffin. Preparation thickness was controlled by supporting slivers of coverslips of known thickness or by Turtox plastic rings (no. 320A196; General Biological Supply House, Chicago, Illinois). Microchambers for all horizontal studies were made after the method of Dellinger (1906), using 2 × 2 and 1 × 2 in. coverslips for sides and the flat edge of a microscope slide fragment as a base.

All cinematographic recordings were made with an Artiflex 16-mm ciné camera driven by a synchronous motor through gear trains for 8 and 16 frames per second (f.p.s.), or through a 'DOM' animation device (Arriflex Corporation, New York, New York) for 2 and 1 f.p.s. or for longer periods of time-lapse recording. The film of choice for recording locomotion and axopodial behaviour was High Contrast Pan (Kodak Ltd., London, England), an extremely fine-grain negative film. Its rated speed (ASA 4–12) was increased about eightfold by development in Diafine (Bauman Chemical Corporation, Chicago, Illinois). Sixteen-mm ciné prints were analysed with a modified Kodak Analyst (Photo-optical Data Analyzer: L–W Photo Inc., Van Nuys, California). Frame positioning error on projection was less than 0·5 % of the frame length.

RESULTS

Actinosphaerium eichhorni and *Actinophrys* sp. move with velocities ranging from 5 to 100 μ/min, sluggish speeds compared to those exhibited by other amoebae (Wolpert, 1965). When unimpeded and viewed at low magnification, their locomotion resembles a very slow and erratic dance, a behaviour documented cinematographically for *A. eichhorni* by Kuhl (1951). *Actinophrys* sp. also occasionally displays a backwards-and-forwards rocking motion that does not result in translation (see Penard, 1904).

Actinosphaerium slightly compressed between a slide and coverslip continues to move. If the overlying coverslip restricts the free movements of only some axopods, then locomotion occurs almost as rapidly as normal. An organism actively moving in such a preparation is shown as viewed from above in Fig. 6. The cytosomes of such animals are flattened anteriorly, and their anterior axopods are fewer and shorter than the trailing ones (some of which adhere over most of their length to the upper coverslip). Under such conditions, *Actinosphaerium* would seem to be rolling forward, because leading axopods slowly detach from the overlying coverslip and pass downward and out of focus.

Due to light scattering by the cytosome, the behaviour of axopods passing beneath the organism can only be inferred from vertical observation. However, these supportive axopods could be observed continuously from the side by the technique of Dellinger (1906). Time-lapse ciné records (1 f.p.s.) were made of the locomotion that occurred within a known focal plane.

Frame-by-frame film analysis indicates that *A. eichhorni* and *Actinophrys* sp. roll: for example, in travelling from left to right, their cytosomes revolve in a clockwise direction. (The term 'clockwise' refers to the direction of cytosome rotation *when the organisms are viewed from the side*. If observed from above, the same organisms would

appear to be rolling forward.) The clockwise movement of two fixed points along the cytosomal periphery (unlabelled arrows) of *A. eichhorni* can be seen in Fig. 4A–C. In all instances except one, however, the arc through which a point on the surface of the cytosome moved was considerably shorter than the total displacement of the organism. In Fig. 4, for example, *A. eichhorni* revolved through a 9° arc, a circumferential distance approximately 27 % of its total translation. Additional data for both species are presented in Table 1.

Table 1. *Resolution of heliozoan locomotion into the arc through which each organism rolled and its horizontal translation*

Organism	Time (sec)	Arc		Translation (μ)	Arc/translation (%)
		°	μ		
Actinosphaerium eichhorni					
1 (Fig. 4)	240	9	37·1	135·0	27·0
2	144	8	29·6	75·0	39·5
3	200	17	64·9	180·0	36·0
4	202	8	28·1	37·5	74·9
7	88	8	29·6	52·5	56·4
8	516	10	40·1	93·8	42·8
	Mean arc/translation = 46·7 % (S.D. = 16·9 %).				
Actinophrys sp.					
1	140	9	4·8	21·4	22·4
2	200	8	3·9	18·6	21·0
3	140	17	8·7	18·6	46·8
4	346	11	5·8	25·0	23·0
	Mean arc/translation = 28·3 % (S.D. = 12·3 %).				

During locomotion, leading axopods shorten and thicken, and trailing axopods elongate. In Table 2 and Figs. 1 and 4, axopods labelled *a* and *b* are always trailing; those marked *c* are more or less directly underneath the cytosome; and *d* and *e* are axopods that have most recently come into contact with the substratum and therefore are leading the organism. (If more than two leading or two trailing axopods or one axopod directly beneath the organism were measured, then a subscript was assigned to the appropriate letter, e.g. e_1, c_1 in Table 2.) Note, for example, the changes in axopods *d* and *a* as the sequence progresses (Fig. 4). The lengths of unattached axopods are relatively constant; consequently, the sequential changes in length exhibited by attached axopods as they move under an advancing organism are all the more significant. In Fig. 1, the percentage ratios of the lengths of the indicated attached axopods (*a*, *b*, etc.) to an average length of several unattached axopods are plotted against time. During this sequence, the length of those unattached axopods that were measured varied less than 5 % from their mean. By contrast, leading axopod *e* shortened approximately 20 % of this average length during the first 40 sec, and by the end of the sequence (240 sec), it was approximately 45 % shorter.

Often, trailing axopods continued to lengthen after detachment from the substratum, while leading axopods were not observed to shorten prior to attachment.

The movements of axopodial attachment points relative to the substratum might provide information for localizing the motive forces responsible for locomotion. If the axopod tips were actively moving across the substratum, perhaps by movement of their

Table 2. *Changes in length of certain supportive axopods during locomotion, and the degree to which each moved forward with the organism ('slippage')*

Organism	Translation, μ	Axopod	Axopod length: Before (μ)	Axopod length: After (μ)	Difference: μ	Difference: %	'Slippage' (% of translation)
Actinosphaerium eichhorni							
1 (Fig. 4)	135·0	*a*	206	229	23	11	56
		b	158	176	18	12	28
		c	120	109	−11	−9	31
		d	150	68	−87	−55	25
		e	259	169	−90	−35	28
2	75·0	*a*	206	199	−7	−4	55
		b	161	176	15	9	9
		c	94	82	−12	−12	23
		c_1	98	75	−24	−23	27
		d	94	45	−49	−52	18
		e	169	128	−41	−24	9
		e_1	202	158	−44	−22	23
3	180·0	*a*	150	225	75	50	46
		c	71	83	12	16	4
		d	120	52	−68	−56	−15
		e	221	131	−90	−41	18
4	37·5	*a*	112	112	0	0	83
		c	68	71	3	6	25
		c_1	105	75	−30	−29	8
		d	112	86	−36	−23	8
		e	135	105	−30	−22	16
7	52·5	*a*	199	206	7	4	50
		b	146	146	0	0	29
		c	109	98	−11	−10	57
		d	142	124	−18	−13	43
		e	248	206	−42	−17	0
Actinophrys sp.							
1		*a*	60	67	7	11	
		b	33	36	3	11	
3		*a*	34	34	0	0	
		b	61	63	2	3	

surface, then the distance they traversed should approximate the horizontal displacement of the organism. If, on the other hand, the observed changes in axopodial length were generating the motive force, then the attachment points would be expected to

function as anchors and to 'slip' at most a fraction of the distance traversed. Values of forward motion of attached points relative to displacement of the organism are presented in the last column of Table 2. While some forward movement does occur, it would seem that the axopods, especially certain leading ones, are not actively moving across the substratum.

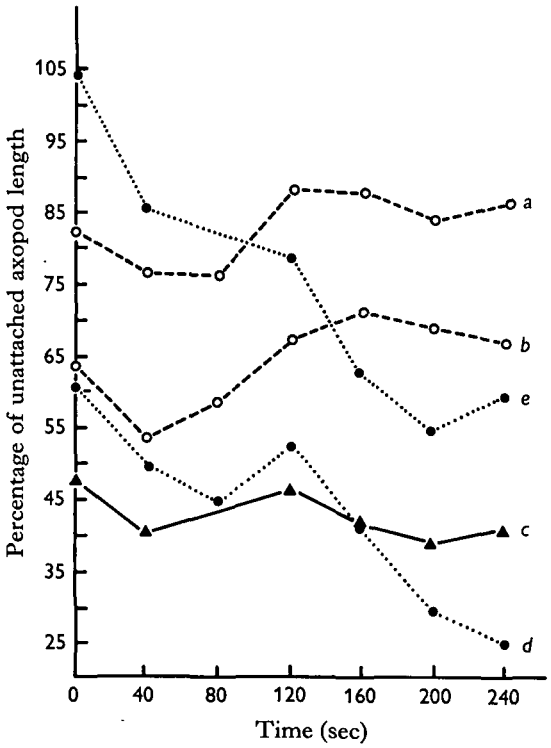

Fig. 1. Temporal variation of the lengths of the five supportive axopods indicated in Fig. 4. The data are expressed as percentages of the mean length of six unattached axopods from the same organism.

When viewed from the side, the lengths of attached axopods of smaller forms (e.g. *Actinophrys*) cannot be measured with the same precision, because of their proximity to the highly refractile glass substratum. Even so, there is some evidence that the attached axopods of motile *Actinophrys* also undergo significant changes in length (Table 2). The highly refractile granules ordinarily localized within the basal portion of each axopod behave in a characteristic manner during locomotion: within shortening axopods, they move into the cytosome proper; within elongating, trailing axopods, they move distally towards the tip.

So far it has not been technically possible to record sequential deformations of a *single* axopod as it attached and then passed completely beneath a moving organism. As noted earlier, heliozoans often change their direction of movement, and frame-by-frame film analyses could only be made of organisms moving within a single focal plane.

In addition to the horizontal and rolling motions indicated in Table 1, these organisms exhibit 'rising' and 'settling' movements in a vertical direction, a phenomenon

first observed for *A. eichhorni* by Brandt (1878). Such movements are strikingly evident in side view, but are usually not observed from above, either because of the larger depth of focus at low magnification or because such vertical motion is restricted by slide and coverslip.

A suitable hypothesis of heliozoan locomotion, therefore, must account for all three directional components (horizontal and vertical movements, and rolling), and should also explain exceptional cases where only one or two such movements are evident.

Three incidental observations should be mentioned since they have some bearing on the role of axopods in locomotion. (i) During cytokinesis, two daughter organisms appear to move actively away from each other (see Kuhl, 1953; Kitching, 1964); they are connected by a cytoplasmic bridge that becomes more and more attenuated until separation occurs. Initially, however, neither half possesses trailing axopods, and in this instance the motive force resulting in separation would have to be localized in either the lateral or the leading axopods. (ii) Specimens of *A. eichhorni* mounted in a thin layer of 0·8 % purified agar (Difco) appear viable for 24 h and continue to move, though much more slowly than is normal. After 5–8 h in such a preparation, motile individuals assume a blunt ellipsoid shape with the major axis parallel to the direction of locomotion. The majority of axopods are trailing the organism; the few leading ones are relatively shorter and much thicker than normal. They are also thicker than the remaining agar-embedded axopods of the same organism. Under these circumstances, it would seem that the leading axopods are responsible for the elongated shape and the movement observed. (iii) *Actinosphaerium* has frequently been observed to climb the vertical glass walls of Dellinger chambers.

DISCUSSION

Heliozoans have been reported to roll, glide or even swim across the substratum (Trégouboff, 1953; Kuhl, 1951), but their motility has not been so critically analysed as has the motility of other sarcodines (see Allen, 1961). It is apparent that the heliozoan axopod is a locomotory appendage, as is the pseudopod of the amoeba, but can the supposedly different types of locomotion be attributed to a single mode of axopodial activity, or must several be invoked?

In the present study, the locomotion of *Actinosphaerium eichhorni* and a species of *Actinophrys* was primarily a horizontal displacement accompanied by definite rolling and vertical motions. This locomotion could be correlated with significant changes in the lengths of certain supportive axopods and seemed to depend on their attachment to the substratum. If, in fact, these changes do result in locomotion, then the motive force so generated would be imparted by the axopods to the cytosome at their points of insertion.

The three components of heliozoan locomotion (Fig. 2, A) could be generated by changes in the lengths of axopods in a simple vectoral manner, and the angle (θ) at which an individual axopod joined the cytosome (Fig. 2, C) would determine the relative magnitude of the three motion components it contributed to the resultant locomotion. In the simplest instance, where an axopod extended along a radial pro-

jection from the approximate centre of gravity, its motive force would generate vertical and horizontal motions (Fig. 2, B). (For this analysis, the cells are considered to be homogeneously dense spheres whose centres of gravity are located at their approximate geometric centres.) A rolling component could be generated by those axopods joining the cytosome at an angle (θ) other than 90°. For an organism being pulled from left to right, this point is illustrated in Fig. 2, C: an angle θ less than 90° will result in

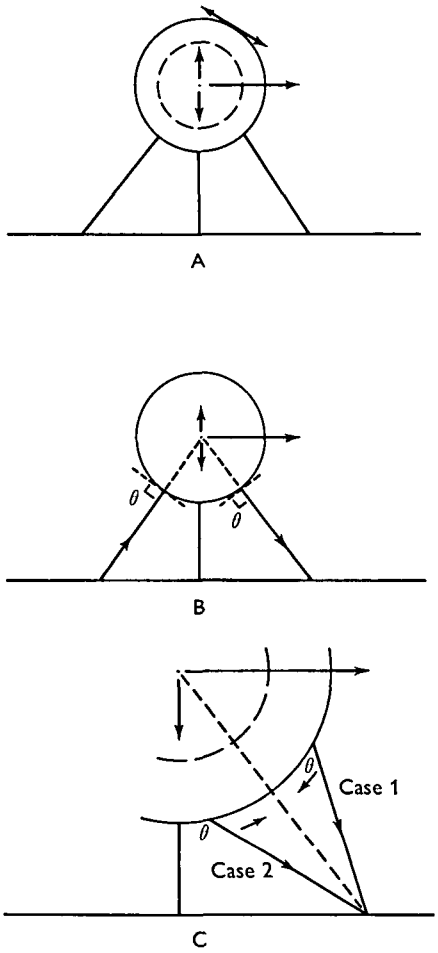

Fig. 2. Idealized profiles of a heliozoan with three supporting axopods. Of the three motion components indicated in A, vertical and horizontal ones would result from pulling and pushing axopods that project radially from the cell's centre of gravity as in B, where $\theta = 90°$. Additional clockwise (case 1) or counterclockwise (case 2) components could be generated by axopods joining the cytosome at angles other than 90° (C).

a clockwise motion (case 1) and a counterclockwise motion from an angle θ greater than 90° (case 2). Penard (1904) briefly indicated that a pulling axopod inserted above the equator would impart a distinct rolling motion to the organism. This expectation also follows from the above analysis (case 1), since an axopod joining the cytosome

above the equator would form an acute angle θ. Conversely, the forcible elongation of a trailing axopod would produce a clockwise motion for an angle greater than 90°, a counterclockwise one for an angle less than 90°.

In all organisms that consistently rolled forward in the direction in which they were moving (case 1), certain leading axopods often joined the cytosome at angles less than 90° (e.g. *d* in Fig. 4). The angle of trailing axopods, however, rarely varied from approximately 90°. Active shortening would seem more important than elongation in the generation of motive force for several other reasons. Shortening axopods generally exhibited a greater rate of change (Table 3) and less slippage (Table 2) than elongating ones. Under certain circumstances, for example, at cytokinesis, trailing (elongating) axopods are not initially present, yet division is accomplished by the active movement of the two daughter halves away from each other.

Table 3. *The rate of change in length of specified axopods during the locomotion of Actinosphaerium eichhorni; the data are collated from Tables* 1 *and* 2

Organism	Elongation (μ/min) Trailing (a, b)	Retraction (μ/min) Directly beneath (c, c_1)	Retraction (μ/min) Leading (d, e, e_1)
1	5·8, 4·5	−2·8	−21·8, −22·5
2	2·9, 6·2	−5·0, −10·0	−21·7, −17·1, −18·3
3	22·8	−3·6	−20·6, −17·1, −18·3
4	0·0	0·9, −9·1	−10·9, −9·1
7	5·4	−8·5	−13·8, −32·3
Average velocity	6·8	−5·7	−19·8

All of the means differ significantly from each other at the $P = 0{\cdot}05$ level (modified Keul's multiple range test).

Rising and settling movements would occur when an organism moved over axopods (in the '*c*' position) that had shortened to varying degrees. Occasionally, upward motions in the absence of horizontal displacements were exhibited by organisms with elongating supportive axopods.

The direction of forward motion would be a resultant of all horizontal motion components, and competition between supportive axopods pulling from different directions would be expected to generate the erratic locomotory behaviour that has been well documented for *A. eichhorni* (Kuhl, 1951). However, locomotion is not always patternless. Specimens of *Actinosphaerium* have been reported to move parallel to one another (Kuhl, 1951), and movement of one organism towards another followed by contact and immediate reversal of movement has also been observed (unpublished observation). Some degree of co-ordination of axopodial behaviour would seem to be present, in spite of the absence of ultrastructural evidence (Tilney & Porter, 1966).

An hypothesis relating heliozoan locomotion to the tensile forces generated by changes in axopodial lengths can account for the phenomena reported in this paper. The hypothesis suggests further that the relative magnitudes of each locomotory com-

ponent could be related to dimensional parameters such as cytosomal diameter and axopodial length. For instance, in Fig. 3 the angle θ discussed above would in turn vary inversely with the angle an axopod made with the substratum (Φ). The locomotion of heliozoans with small cytosomes and relatively long axopods (such as *Actinophrys*) should contain a small rolling component, which possibly might not be detected (Fig. 3, B). Larger organisms, such as *Actinosphaerium*, that possess shorter axopods relative to their large cytosomal diameter should exhibit a greater rolling motion (Fig. 3, A). Though the data are by no means conclusive, the larger *A. eichhorni* seems to roll more per unit of horizontal displacement than does the smaller *Actinophrys* with relatively longer axopods (Table 1).

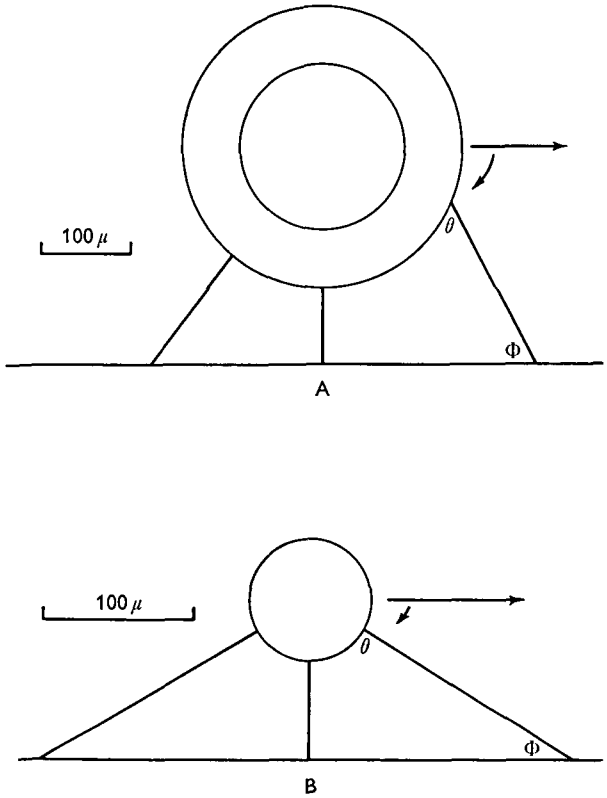

Fig. 3. Idealized profiles of two different species of heliozoans. *Actinosphaerium eichhorni* (A) generally possess a larger cytosome and relatively shorter axopods, while the much smaller *Actinophrys* sp. displays relatively longer axopods (B). For further explanation, see text.

Since cytosomal size and axopodial length are relatively good taxonomic criteria for the species studied here (Penard, 1904), the hypothesis provides a single explanation for the different kinds of locomotion that have been reported for these as well as other species. If not carefully examined, heliozoans such as *Actinophrys* (Fig. 3, B) might seem simply to be 'gliding' or 'creeping', while those similar in dimensions to *Actinosphaerium* (Figs. 3, A and 4) would appear to be 'rolling' across the substratum.

Yet, in both instances, changes in axopodial length would provide the necessary motive force.

Other possible axopodial mechanisms have been proposed and their variety reflects, in part, the differences in the types of locomotion observed: for example, whether the organism was 'rolling' or 'gliding'. Although the hypothesis proposed in this paper could account for the apparent observational discrepancies, the explanatory mechanisms will be examined in more detail.

Conceivably, changes either in the length of axopods or in their position relative to the substratum could result in locomotion. This study presents the first quantitative data relating locomotion with changes in axopodial length, although the idea that attached axopods might pull a heliozoan forward has been discussed before (Penard, 1889). Sequential shortening of supportive axopods might also generate a 'rolling' motion if these axopods were asymmetrically located relative to the organism's centre of gravity (Wetzel, 1926). Such a hypothesis, similar to one suggested more recently by Tilney & Porter (1966), proposes that locomotion would result more from a shift in the cell's centre of gravity than from tensile forces generated by axopodial shortening. Since the organism would be rolling forward, the arc through which it moved should equal its horizontal displacement, or even slightly exceed it (allowing for slippage), and substantial adhesion of the axopods to the substratum should not be necessary. The observations and results presented here do not support such an explanation. In no instance was the arc through which an organism rolled greater than 75 % of its horizontal displacement, and in most instances it was less than 50 %. The motive force generated by slight shifts in the centre of gravity should be small, and consequently slightly compressed organisms should roll with great difficulty or not at all. In fact, locomotion continued under mild compression, even though the movements of overlying axopods was obviously impeded. Finally, locomotion of a heliozoan up a vertical surface cannot result simply from a shift in the organism's centre of gravity.

Changes in axopodial position seem to be of minor importance in locomotion, although their possible significance cannot be completely discounted. Based on time-lapse cinematography, Kuhl (1951) ascribed the locomotion of *A. eichhorni* to the 'rowing movement' (Ortsbewegung) of lateral axopods that transcribed arcs of 8–12°. As Tilney & Porter (1966) have more recently noted, locomotion would result from such rowing motions if the axopods either display a differential speed between effective and recovery strokes, or attach to the substratum during their effective stroke and detach during recovery. (This latter motion might be more aptly described as 'walking'.) Rowing motions were rarely observed in this study and were never symmetrically organized relative to the axis of locomotion. As a consequence, it is difficult to visualize how directed movements, even of brief duration, could result from such behaviour, especially in those instances where a slightly compressed organism continued to move. More often, axopods displaying rowing motions with differing effective and recovery speeds were localized near contractile vacuoles that gradually filled and rapidly collapsed.

Kitching (1964) also doubts that axopodial rowing movements are responsible for locomotion. Instead, he suggests that the locomotion of *Actinophrys sol* might result

from the surface movements of attached axopods. Movement of this cytoplasmic surface layer, independent of gross changes in the length of the axopods themselves, seems to be implicated in the rejection of material and possibly in feeding (Kitching, 1960, 1964). Attached axopods, moving the organism by means of such surface movements, should advance at a rate similar to that of the whole organism. Such behaviour was not observed. In both species studied, the tips of leading axopods, rather than advancing, seemed to function as anchors and all attached axopods observed either underwent a change in length or were bent relative to the direction of locomotion.

Finally, attached axopods might propel a heliozoan by active bending (Tilney & Porter, 1966). Bending of attached axopods that are obliquely oriented relative to the direction of locomotion is a commonly observed phenomenon (Fig. 6), but it is difficult to decide whether such bending is a cause or an effect of locomotion. The data presented here neither affirm nor negate the importance of bending axopods in locomotion; however, two further observations are perhaps suggestive of their derivative nature. Only attached axopods of moving organisms bend along their length to any great extent; freely suspended axopods are straight and relatively stiff and one has the impression they are passively bent by large motile prey and also by the substratum. However, both attached and unattached axopods exhibit striking changes in length. Axopodial shortening serves an important feeding role (Looper, 1928; Watters, 1966) and, as has been shown here, significant changes in the lengths of attached axopods can be correlated with locomotion. Further, the rates of change in length exhibited by these attached axopods are similar to those exhibited by unattached ones (Watters, 1966).

Changes in pseudopod lengths are responsible for the locomotion of at least three other types of cells: *Difflugia*, a test-bearing sarcodine (Wohlman & Allen, 1968), mesenchyme cells of sea-urchin gastrulae (Gustafson & Wolpert, 1963), and sensory neurons in culture (Nakai, 1964). In these forms, the ultrastructural basis for the changes in length has not been adequately characterized, although the work of Wohlman & Allen (1968) strongly implicates 50-Å microfilaments in the contraction of the testacean pseudopod. In the case of the Heliozoa, on the other hand, locomotion would seem to depend on the integrity of the microtubular elements of the axoneme and the structural support they contribute to the axopod. Treatments, such as low temperature, colchicine and high hydrostatic pressure, have been shown to affect adversely both the stability of axopods and the structure of microtubules (Tilney, 1965; Tilney *et al.* 1966). Axopods also contain a highly motile cytoplasmic layer that is continuous with the equally motile cytosomal surface. In *Actinosphaerium eichhorni* this latter cytoplasmic layer rapidly forms food cups and, under certain circumstances, extensive cytoplasmic veils (Penard, 1904; Watters, 1966). An understanding of the relationship between locomotion and this associated cytoplasmic motility would contribute greatly to our understanding of the dual role proposed for the microtubule in structural support and motility.

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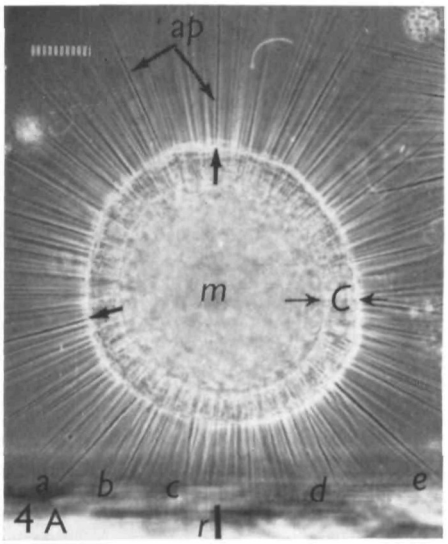
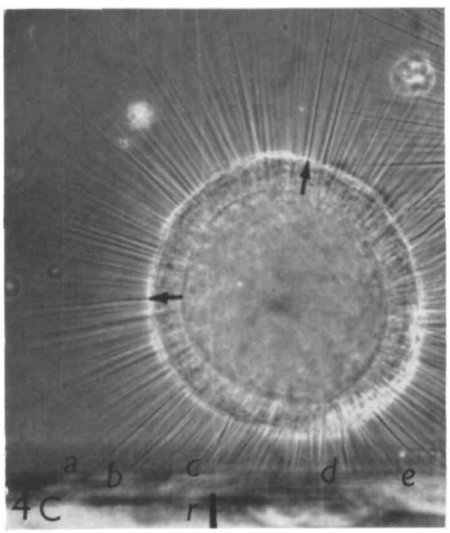
In all Figures, each small scale unit represents 10 μ.

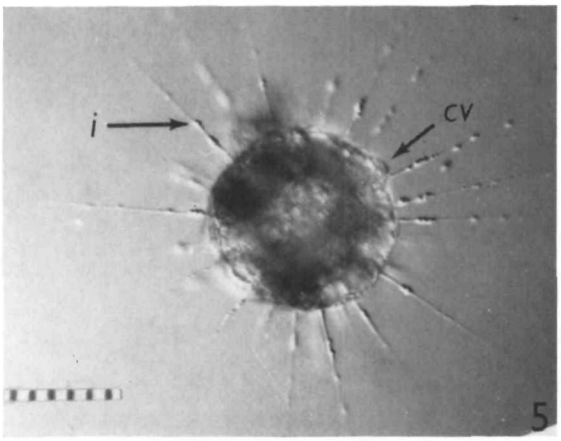
Fig. 4. Prints from a 16-mm ciné record of the locomotion of *Actinosphaerium eichhorni*, as viewed from the side, at the following time intervals: A, 0 sec; B, 160 sec; C, 240 sec. *A. eichhorni* possesses numerous axopods (*ap*) and a cytosome characteristically divided into a vacuolar cortex (*C*) and a more opaque medullary region (*m*). As the organism moves from left to right, supporting axopods (*a–e*) undergo chracteristic changes in length. The two unlabelled arrows along the periphery indicate the same two axopods throughout the sequence, while the solid bar (*r*) provides a fixed reference mark on the substratum.

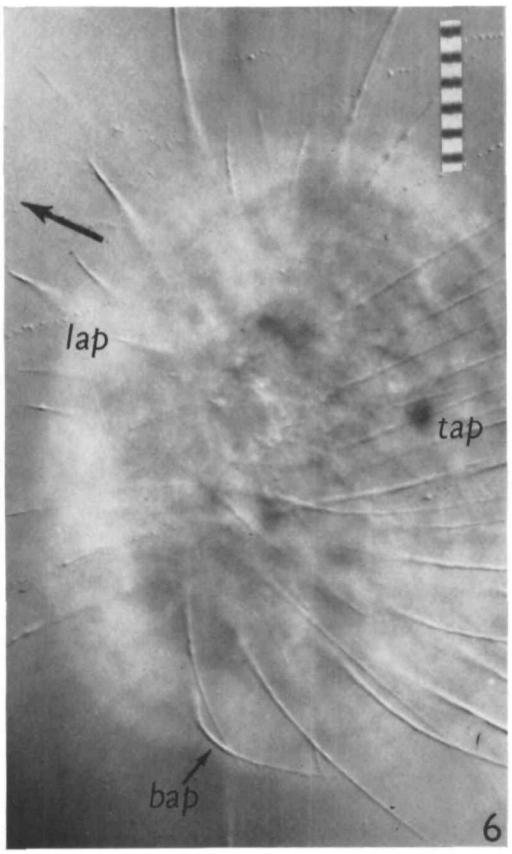
Fig. 5. *Actinophrys* sp. displays a typically spherical cytosome and radiating axopods. Numerous inclusions (*i*) within the axopods and also a 'contractile vacuole' (*cv*) are obvious at this magnification.

Fig. 6. A slightly compressed *A. eichhorni* that has continued to move in the direction of the large arrow. A few short, leading axopods (*lap*) are attached at their tips to the overlying coverslip, while numerous trailing axopods (*tap*) are attached along their entire length. A laterally located bent axopod (*bap*) can also be seen. The very narrow depth of field achieved both in this figure and in Fig. 5 results from use of the Nomarski Differential Interference system (Carl Zeiss).









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