

Fig. 2. Calcium uptake of rabbit aorta microsomal vesicles in a representative experiment. Incubation at 37°C is described in text. (Curve a) 100 μ M calcium, complete medium; (curve b) 20 μ M calcium, complete medium; (curve c) 100 μ M calcium, oxalate omitted; (curve d) 100 μ M calcium, Mg-ATP omitted.

ammonium oxalate, 15 μ mole; sodium azide, 15 μ mole; $^{45}\text{CaCl}_2$, 0.4 μ c; CaCl_2 , 0.3 or 0.06 μ mole; and 0.2 ml of the microsomal or mitochondrial fraction. We determined the uptake of radioactive calcium by the vesicles by filtering samples of the particles in the incubation medium, washing them on a Millipore filter, and counting them in a liquid scintillation counter (2).

Several features that characterize the uptake of calcium by the microsomal vesicles are shown in Fig. 2. In the first minute a rapid initial binding is observed that does not require the presence of Mg-ATP. Further uptake of calcium is dependent on Mg-ATP. The magnesium salt of adenosine diphosphate (Mg-ADP) cannot be substituted for Mg-ATP. Calcium uptake is greatly enhanced by the addition of 5 mM oxalate and is not inhibited by the addition of 5 mM azide. The rate of calcium uptake is dependent on the calcium concentration and is higher at pH 7.4 than at pH 6.8. No active uptake is seen at 2°C.

The uptake of calcium by the mitochondrial fraction requires the presence of oxalate and Mg-ATP or a substrate such as α -ketoglutarate and Mg-ADP. The net uptake of calcium by mitochondria is approximately 25 percent of that found with the microsomal preparation. The mitochondrial system is almost completely inhibited by the addition of 5 mM azide.

Rabbit aorta is made up of several

layers of tissue that are mechanically separable (3). The adventitial layer is composed of connective tissue and some smooth muscle. The medial-intimal layer is composed primarily of vascular smooth muscle with an endothelial layer as a minor component. When these two layers were separated and examined, the total microsomal calcium uptake was 18 percent in the adventitial and 82 percent in the medial-intimal layer. The implication is that the active calcium uptake is associated with microsomes derived from smooth muscle cells of the vascular tissue.

The characteristics of the calcium uptake system of the aortic microsomal vesicles are similar to those described for microsomes from skeletal and cardiac muscle (4). Active uptake of calcium in uterine smooth muscle has also been characterized (5).

An adenosine triphosphatase activity dependent on magnesium plus calcium is associated with skeletal muscle microsomes and is large enough to potentially relate to the calcium sequestration system (6). With vascular smooth muscle the extra ATP splitting caused by incubating microsomes and 20 μ M calcium in the presence of 5 mM Mg-ATP for 20 minutes is 0.4 μ mole of inorganic phosphate (P_i) per milligram of microsomal protein. This is a low level of activity, but it is more than sufficient to suggest a relation between the calcium uptake and the ATP hydrolysis.

The microsomal calcium extrusion system could be important in the regu-

lation of vascular tone. The apparent quantitative capacity of the aorta to extrude calcium through its microsomal system appears to be adequate. There is approximately 3.3 mg of microsomal protein for each gram of aortic tissue, which probably would contain no more than 8 nmole of cytoplasmic calcium (10^{-5}M). Physiologic factors governing the entry of calcium into the cytoplasm also modulate the contractile state of the muscle fibers. The isolation and further purification of microsomal vesicles from vascular tissue may permit a direct examination of the manner in which vascular tone is regulated by agents that affect the uptake or release (or both) of calcium in subcellular systems.

DAVID F. FITZPATRICK, ERWIN J. LANDON
GAMIL DEBBAS, LEON HURWITZ
Department of Pharmacology,
Vanderbilt University School of
Medicine, Nashville, Tennessee 37203

References and Notes

1. D. F. Bohr, *Pharmacol. Rev.* **16**, 85 (1964); E. E. Daniel, in *Muscle*, W. M. Paul *et al.*, Eds. (Pergamon, New York, 1965), p. 295; A. P. Somlyo and A. V. Somlyo, *Pharmacol. Rev.* **20**, 197 (1968); L. Hurwitz and A. Suria, *Annu. Rev. Pharmacol.* **11**, 303 (1971).
2. R. F. Palmer and V. A. Posey, *J. Gen. Physiol.* **55**, 89 (1970).
3. R. A. Maxwell, S. B. Eckhardt, W. B. Wastila, *J. Pharmacol. Exp. Ther.* **161**, 34 (1968).
4. A. Martonosi and R. Feretos, *J. Biol. Chem.* **239**, 684 (1964); A. M. Katz and D. I. Repke, *Circ. Res.* **21**, 153 (1967).
5. M. E. Carsten, *J. Gen. Physiol.* **53**, 414 (1969).
6. A. Martonosi and R. Feretos, *J. Biol. Chem.* **239**, 659 (1964).
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Sea Star Platasterias:

Ossicle Morphology and Taxonomic Position

Abstract. *The modern sea star Platasterias latiradiata, on the basis of ossicle morphology, is removed from the fossil subclass Somasteroidea and assigned to the genus Luidia of the living subclass Asterozoidea. A somasteroid assignment of this species should not be used in inferences concerning evolution and morphology of primitive echinoderms.*

Spencer (1) recognized the lower Paleozoic asterozoan subclass Somasteroidea as an extremely primitive ancestral group which arose at an early stage in the development of echinoderm lineages. Fell (2) considered the shallow water Central American sea star *Platasterias latiradiata* Gray, 1871, to be a living representative of the somasteroids, and he drew certain phylogenetic inferences from the morphology of this sea star. Madsen (3), on the basis of a comparison of the overall

morphology of *Platasterias* with that of the fossil somasteroids, questioned Fell's ideas, but comparisons of the detailed morphology of discrete ossicles have not been made. Through such comparisons, *Platasterias* is here aligned with *Luidia* Forbes, 1839, as suggested by Madsen; inferences about somasteroid soft parts and asterozoan phylogeny, therefore, should not depend on assignment of *Platasterias* to the Somasteroidea.

Döderlein (4) recognized four super-subgeneric "groups" in his study of the

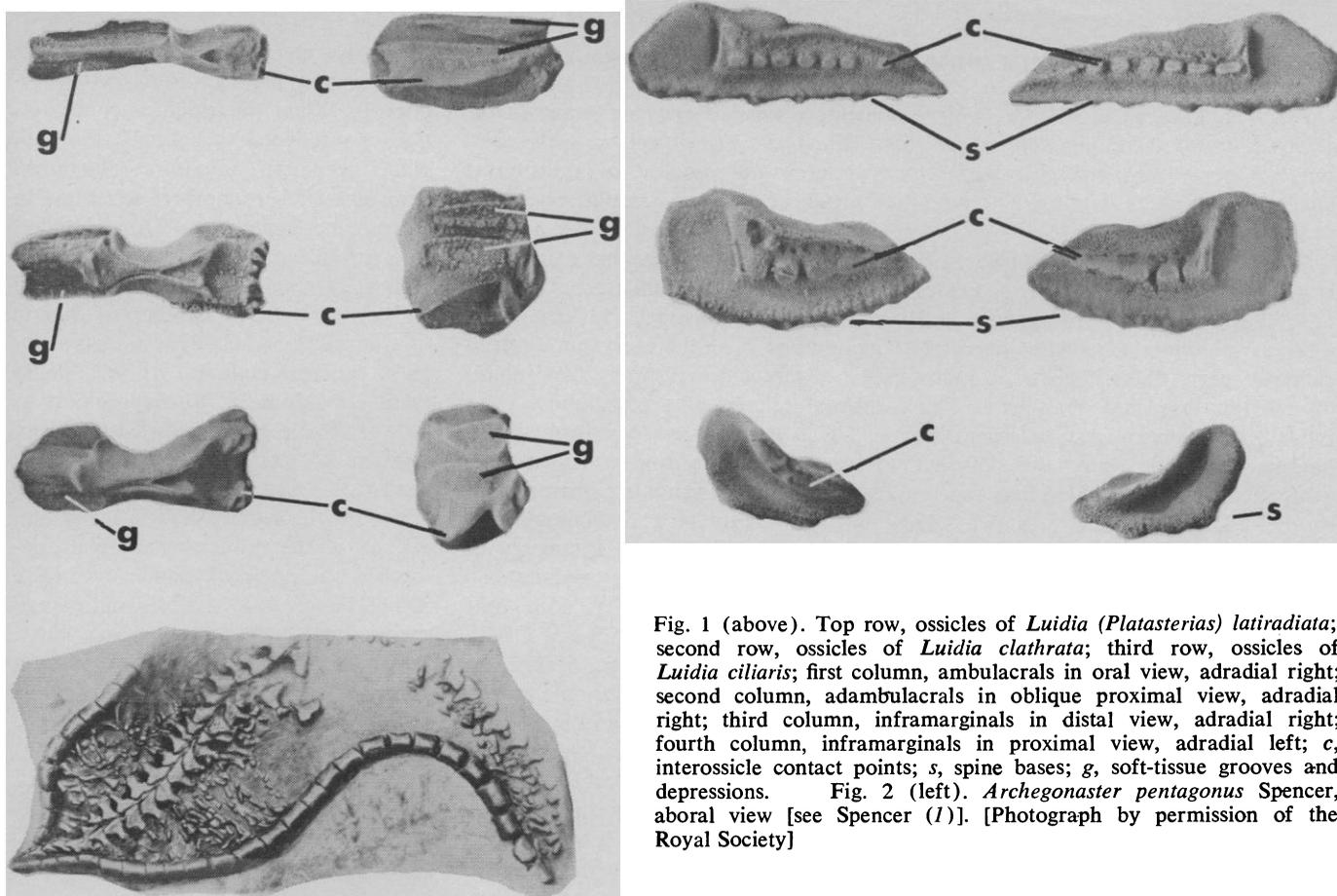


Fig. 1 (above). Top row, ossicles of *Luidia (Platasterias) latiradiata*; second row, ossicles of *Luidia clathrata*; third row, ossicles of *Luidia ciliaris*; first column, ambulacrals in oral view, adradial right; second column, ambulacrals in oblique proximal view, adradial right; third column, inframarginals in distal view, adradial right; fourth column, inframarginals in proximal view, adradial left; c, interossicle contact points; s, spine bases; g, soft-tissue grooves and depressions. Fig. 2 (left). *Archegonaster pentagonus* Spencer, aboral view [see Spencer (1)]. [Photograph by permission of the Royal Society]

genus *Luidia*; for comparative purposes, ossicles from the proximal portions of the arms of members of two of these groups are illustrated in Fig. 1. Member species of any of the four groups display a high degree of similarity to one another in ossicle morphology, but they are, to varying degrees, distinct from members of other groups (5).

In Fig. 1, certain ossicles of *Luidia (Platasterias) latiradiata* are illustrated (top row), with those of *Luidia (Petalaster) clathrata* (Say), 1825, of the Clathrata group of Döderlein (middle row), and *Luidia ciliaris* (Philippi), 1837, of the Ciliaris group of Döderlein (bottom row). Most prominences are interossicle contact points (Fig. 1, c) or spine bases (Fig. 1, s); most grooves and depressions (Fig. 1, g) bear muscle or other soft tissue in the living animal.

The only significant differences between the *Platasterias* ossicles and those of *L. clathrata* are differences of proportion; the ossicles and arms of *Platasterias* are proportionately wider and lower than the corresponding ossicles and arms of *L. clathrata*. Distal ossicles of the two species are similar in proportion as well as morphology because the arms of the two species distally are of

comparable proportions. Ossicles of *L. ciliaris* are distinctive in shape and proportion. Differences in ossicle morphology between *Platasterias* and *L. clathrata*, therefore, are less significant than differences between species assigned to different groups (sensu Döderlein) of *Luidia*. Ossicle morphology of fossil species assigned to the somasteroids is very different from that of *Platasterias* and *Luidia* [for example, see Fig. 2 and (1)].

The detailed morphology of the ossicles, therefore, supports Madsen's (3) inferences of close relationships between *Platasterias* and *Luidia* and his suggestion that the proportions of *Platasterias* arms and ossicles have become relatively transversely elongate in adaptation to life on a shifting sandy bottom and possibly to mode of food attainment. I agree with Madsen that *Platasterias* should either be assigned to the subgenus *Petalaster* (with *L. clathrata*) or be considered a subgenus of its own (within the Clathrata group), as is done here.

Platasterias is recognized as a separate subgenus within the Clathrata group because of its distinctive overall form. Higher taxonomic categories follow Spencer and Wright (6). Fell (2)

elevated certain of the subgenera of Döderlein to the generic level. This change is not followed here; I think, on the basis of ossicle morphology, that three of the four super-subgeneric groups of Döderlein are very closely related and that assignment of these species to a single genus most accurately reflects the relationships of the species.

Subclass Asteroidea de Blainville, 1830
Order Platysterida Spencer, 1951
Family Luidiidae Verrill, 1899
Genus *Luidia* Forbes, 1839
Luidia (Platasterias) latiradiata
(Gray), 1871

D. BRYAN BLAKE

Department of Geology,
University of Illinois at
Urbana-Champaign, Urbana 61801

References and Notes

1. W. K. Spencer, *Phil. Trans. Roy. Soc. London Ser. B* 235, 87 (1951).
 2. H. B. Fell, *Science* 136, 633 (1962); *Paleontol. Contrib. Univ. Kans.* 6 (1962); *Phil. Trans. Roy. Soc. London Ser. B* 246, 381 (1963).
 3. F. J. Madsen, *Nature* 209, 1367 (1966).
 4. L. Döderlein, *Siboga Exped.* 46b, 193 (1920).
 5. D. B. Blake, in preparation.
 6. W. K. Spencer and C. W. Wright, in *Treatise on Invertebrate Paleontology*, R. C. Moore, Ed. (Univ. of Kansas Press, Lawrence, 1966), part U, vol. 1.
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