Edwards *et al.* (*5*) have suggested that digyny (fertilization of a diploid ovum) is probably the more common mechanism involved in man and they present blood group data as evidence. Penrose and Delhanty (*6*) reported a triploid fetus to be derived from one sperm and an unreduced ovum; however, they present no real evidence for this interpretation.

Statistical analyses of sex chromosome distribution (*7*) support diandry because the reported ratio of 17 XXX : 30 XXY approximates the expected ratio 1 : 2. The very low frequency of XYY triploids (4 cases) suggests that strong selective forces may be at work if dispermy, rather than diploid sperm, is the more common mechanism. There is proof that polyspermy does occur in the rat (*1*). An investigation of the DNA content of sperm obtained from men attending a subfertility clinic (*8*) revealed a significant proportion of diploid sperm and indicated that these sperm could be a cause of triploid embryos.

In the case reported here, delayed fertilization may have allowed penetration of the ovum by more than one sperm. However, a period of 13 months without oral contraceptives appears to be sufficient time for recovery of normal function. In the three triplet sets, Nos. 3, 13, and XXY, where the two paternal homologs are different both are present in the triploid cells. This adds to the possibility that the paternal contribution was a diploid gamete formed by both genomes. When markers on more chromosomes are identified, it should be possible to determine which of the two mechanisms is more commonly involved.

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References and Notes

1. C. R. Austin and A. W. H. Braden, *Nature* **172**, 82 (1953).
2. D. H. Carr, *J. Med. Genet.* **8**, 164 (1971).
3. ———, *Can. Med. Ass. J.* **103**, 343 (1970).
4. C. C. Lin, I. A. Uchida, E. Byrnes, *Can. J. Genet. Cytol.* **13**, 361 (1971).
5. J. H. Edwards, C. Yuncken, K. E. Rushton, S. Richards, U. Mittwock, *Cytogenetics* **6**, 81 (1967).
6. L. S. Penrose and J. D. A. Delhanty, *Lancet* **I-1961**, 1261 (1961).
7. A-M. Schindler and K. Mikamo, *Cytogenetics* **9**, 116 (1970).
8. A. T. Sumner, *Nature* **231**, 49 (1971).
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A Calcium Pump in Vascular Smooth Muscle

Abstract. *A microsomal cell fraction derived from the intimal-medial layer of rabbit aorta takes up calcium in the presence of magnesium and adenosine triphosphate. The rate of uptake of calcium is slower than that observed in skeletal muscle microsomes. Uptake of calcium by mitochondria from the aorta is even more limited and, unlike microsomal uptake, is inhibited by azide.*

The mode of regulation of vascular muscle tone is of medical importance. At the cellular level the agent that ultimately controls the mechanical activity of smooth muscle fibers is the calcium ion (*1*). Isolation of subcellular units that are involved in the transport of calcium into and out of the cytoplasm is useful for elucidating mechanisms by which vascular tone is regulated in health and disease. Two energy-dependent calcium sequestration systems have now been isolated from the smooth muscle of the rabbit aorta.

We prepared subcellular fractions of rabbit aorta by homogenizing approximately 1.5 g of the freshly excised vascular tissue in ten volumes of a cold (0 to 2°C) isotonic sucrose medium by means of a Potter homogenizer fitted with a Teflon pestle. We prepared the microsome fraction by centrifuging the homogenate at 1500*g* for 10 minutes, the supernatant at 27,000*g* for 10 minutes, and the new supernatant at 105,000*g* for 60 minutes. The final pellet was resuspended in 2 ml of isotonic sucrose for immediate use. Only fresh microsomal preparations were used. We prepared mitochondria by centrifuging the homogenate for 10 minutes at 1500*g* and the supernatant for 10 minutes at 9500*g*. This pellet was also resuspended in 2 ml of isotonic sucrose for immediate study.

Figure 1 shows an electron micrograph of the microsomal pellet. The fraction consists of vesicular structures enclosed, for the most part, by smooth membrane but with an occasional vesicle enclosed by rough endoplasmic reticulum. No intact mitochondria were encountered, and the preparation is free of succinate dehydrogenase, which served as a biochemical indicator of the presence of mitochondrial membranes.

Calcium uptake was studied at 37°C in a 3-ml incubation mixture containing tris(hydroxymethyl)aminomethane (tris-HCl, *p*H 7.4), 30 μmole; KCl, 300 μmole; the magnesium salt of adenosine triphosphate (Mg-ATP), 9 μmole;

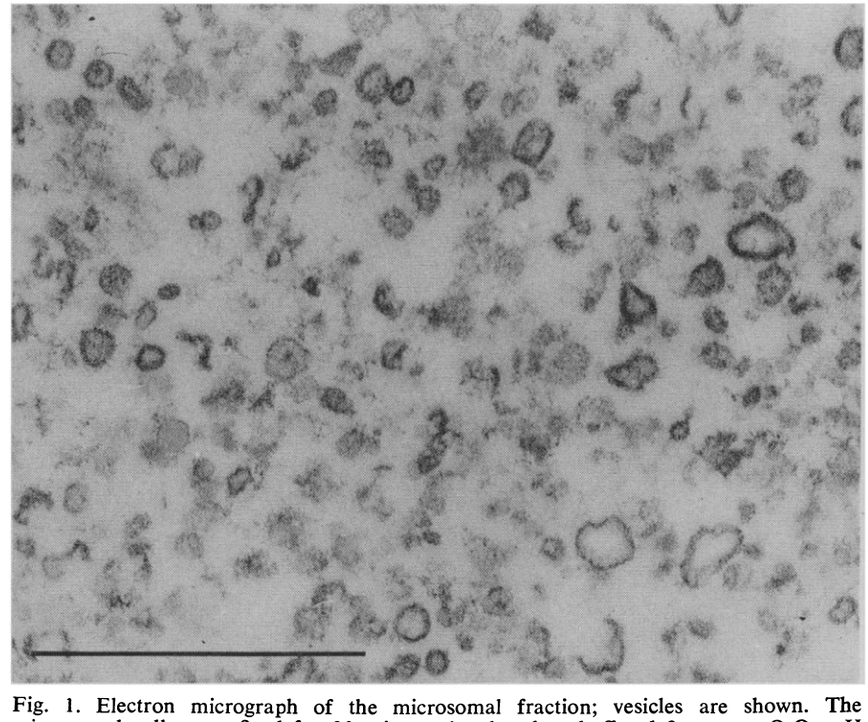

Fig. 1. Electron micrograph of the microsomal fraction; vesicles are shown. The microsomal pellet was fixed for 90 minutes in phosphate-buffered 2 percent O_8O_4, *p*H 7.5. The pellet was then dehydrated in ethanol and propylene oxide and embedded in araldite. The sections were stained in uranyl acetate and lead citrate. The scale marker is 0.5 μm.

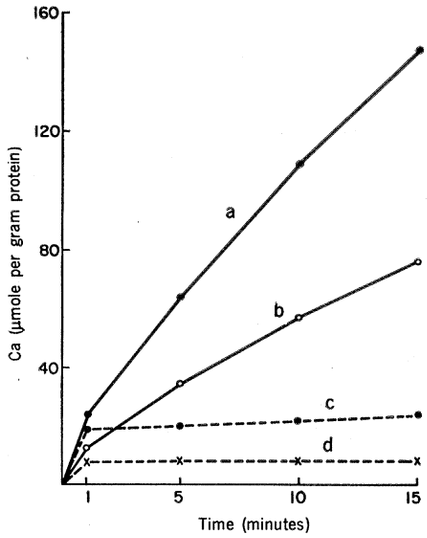

Fig. 2. Calcium uptake of rabbit aorta microsomal vesicles in a representative experiment. Incubation at 37°C is described in text. (Curve a) 100 μM calcium, complete medium; (curve b) 20 μM calcium, complete medium; (curve c) 100 μM calcium, oxalate omitted; (curve d) 100 μM calcium, Mg-ATP omitted.

ammonium oxalate, 15 μmole; sodium azide, 15 μmole; $^{45}CaCl_2$, 0.4 μc; $CaCl_2$, 0.3 or 0.06 μmole; 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 *p*H 7.4 than at *p*H 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 μM calcium in the presence of 5 mM Mg-ATP for 20 minutes is 0.4 μmole 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 regulation 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.

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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).
7. Supported by PHS grant AM-04703 and by NSF grant GB-13346.

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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 Asteroidea. 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 supersubgeneric "groups" in his study of the