

Maintenance of fluid volume in the starfish water vascular system

LOCOMOTION in the starfish *Asterias forbesi* involves many tube feet, each functioning independently as a hydrostatic skeleton; the circular muscles of the ampulla acting antagonistically to the longitudinal muscles of the tube foot itself through the constant volume of fluid contained in the ampulla-foot unit¹. The fluid for each tube foot comes from the water vascular system to which each foot is connected through its own lateral canal. The water vascular system in *Asterias* consists of three interconnecting series of canals: (1) radial canals running the length of each arm; (2) a circular canal running around the gut at the base of the arms, and (3) the stone canal which runs from the radial canal up to the aboral ("dorsal") surface, terminating in the madreporite. The madreporite, an orange disk, is porous and associated with several sets of cilia. For some time it has been presumed, and is still presented or indicated in some textbooks, that the fluid contained within the starfish water vascular system is pumped by ciliary activity through the madreporite into the canal system, although it has been pointed out that no experimental evidence supports this assumption². Ionically, the fluid within the water vascular system is nearly identical to the external seawater with the exception of internal K⁺, which is present in a concentration up to 60% higher than that of the seawater^{3,4}. On the basis of this difference, it was suggested that K⁺ accumulation by the water vascular system is responsible for water uptake by this system, either by creating a slight osmotic gradient within the tube feet or by direct movement of water with hydrated K⁺ ions, as opposed to direct uptake of seawater through the madreporite⁵. We have investigated the generation of the fluid in the water vascular system more thoroughly by determining the osmotic and ionic characteristics of the fluid within the tube feet and the ionic transport characteristics of the isolated tube foot epithelium.

We used *A. forbesi* collected in Jamestown, Rhode Island and maintained for up to 1 week before use in tanks of artificial seawater at 7 °C. Fluid from the water vascular system was obtained from an intact starfish by placing it on its aboral surface and tying off individual tube feet close to their base with silk thread. The foot was then isolated from the animal, washed quickly in distilled water, blotted dry and cut open. The released fluid was taken up into a micropipette. Na⁺ and K⁺ concentrations in the tube foot fluid were determined by flame photometry, and Cl⁻ was measured by a modified microelectrometric titration technique⁶, and the total osmolality of the fluid was obtained with a Clifton nanolitre osmometer. These determinations are summarised in Table 1. The Na⁺ concentration of the tube foot fluid was identical to that of the external medium, while internal Cl⁻ was slightly but significantly higher than environmental Cl⁻. In agreement with earlier measurements on other echinoderms^{3,4}, the K⁺ concentration of the tube foot fluid from *A. forbesi* was considerably higher than that of the external seawater. In addition, the tube foot fluid was distinctly hyperosmotic to the surrounding seawater, by up to 22 mosM. The perivisceral (coelomic) fluid was osmotically and ionically (Na⁺, K⁺ and Cl⁻ ion concentrations) identical with the external seawater.

To investigate whether or not the tube foot epithelium itself is in part responsible for K⁺ secretion, individual tube feet were cut off at the base, filled with fresh seawater and tied off with silk thread. Thus initially identical ion concentrations were present on both sides of the isolated tube foot epithelium. The tube foot-sac preparation was allowed to equilibrate in aerated seawater for 90 min.

Analysis then showed that, as in the intact tube foot, the K⁺ concentration of the fluid in the isolated tube foot was higher than that of the surrounding medium. The internal K⁺ concentration was 16.8 mM, while that in the external seawater was 9.5 mM. The secretion of K⁺ by the isolated tube foot epithelium could be inhibited by addition of CN⁻ (1×10^{-4} M) to the external medium. The isolated tube foot could also be set up as a tubular system and perfused⁷; in this manner unidirectional K⁺ fluxes, using ⁴²K (New England Nuclear), could be measured. Unidirectional K⁺

Table 1 Osmotic and ionic characteristics of fluid from intact starfish tube feet and of surrounding seawater

Ion concentration (mmol kg ⁻¹)	Seawater	Tube feet fluid
Na ⁺	469 ± 2.9 (17)	471.4 ± 2.6 (17)
K ⁺	9.5 ± 0.8 (21)	17.3 ± 1.8 (21)
Cl ⁻	543.3 ± 2.6 (19)	551.7 ± 3.1 (19)
Total osmolality (mosmol l ⁻¹)	1,095 ± 3.3 (25)	1,117 ± 3.8 (25)

Figures are mean ± s.e.m., with number of determinations in parentheses.

influx was 4.2×10^{-9} mol cm⁻² min⁻¹ and unidirectional K⁺ efflux was 3.4×10^{-9} mol cm⁻² min⁻¹.

Connection of the tube feet with the outside medium through the canals of the water vascular system and the madreporite was investigated by equilibrating intact starfish for 24 h in seawater with ¹⁴C-polyethylene glycol (New England Nuclear); molecular weight 4,000. Samples of fluid were then removed from the tube feet as before and counted in a liquid scintillation counter. Negligible amounts of label were found inside the tube feet, indicating that no direct connection exists between the fluid in the tube feet and the outside medium across the madreporite, an observation consistent with studies performed with dyes⁵.

None of these observations supports the assumption that the fluid in the tube feet of the starfish is simply seawater pumped into the water vascular system through the madreporite by the action of cilia. The fluid in the tube feet, as well as that in the connecting water vascular system, is higher in K⁺ and Cl⁻, and is hyperosmotic with respect to the external seawater. This hyperosmotic condition is probably brought about by the active secretion of K⁺, with Cl⁻ following passively, across the tube foot epithelium. Other epithelial structures in addition to the tube foot epithelium itself in the starfish may contribute to total K⁺ secretion into the water vascular system, for example, the ampulla epithelium. The generation of a slightly hyperosmotic condition in the tube foot lumen by solute secretion would create a driving force for water across the tube foot epithelium into the tube foot lumen. This water provides the fluid through which the circular muscles of the ampulla and the longitudinal muscles of the foot are antagonised. In addition, water uptake in this fashion could make up for water losses through ultrafiltration across contracted tube feet and through damaged tube feet.

The lack of any direct connection of the madreporite with the more distal portions of the water vascular system in the starfish does not rule out any exchange whatsoever between different parts of the water vascular system and the external seawater through the madreporite. The water vascular system in some echinoderms is probably connected entirely to the outside environment through the madre-

porite. Water movements which do occur through the madreporite in asteroids may serve either a sensory role or could serve to equalise pressure differentials⁸ between the water vascular system and the outside environment.

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