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SOLUTE SECRETION BY THE TUBE FOOT EPITHELIUM IN THE STARFISH *ASTERIAS FORBESI*

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SUMMARY

1. The K^+ and Cl^- levels and osmotic concentration of the tube feet fluid in intact starfish (*Asterias forbesi*) are higher than that in the external sea water or perivisceral (coelomic) fluid.

2. The increase in K^+ in the fluid in the tube feet lumen is brought about by the active secretion of K^+ by the tube feet epithelium.

3. No exchange of material from the external environment with the more distal portions of the water vascular system across the madreporite was observed in this study.

4. It is suggested that the fluid volume in the tube feet is maintained by active K^+ secretion, by the tube feet epithelium, which creates an osmotic driving force for water into the lumen of the tube feet.

INTRODUCTION

The echinoderms are characterized in part by the possession of a unique structure, the water vascular system. In the asteroids, the water vascular system (Fig. 1) consists of a series of canals which are apparently connected to the outside sea water medium through the madreporite and, internally, to the tube feet. The tube feet of the starfish serve a variety of functions, which include locomotion, feeding and respiration. Specialized tube feet may also have a sensory function. An individual tube foot operates as an independent hydraulic unit through the fluid medium contained within the foot-ampulla system. The circumferential muscles of the ampulla act in an antagonistic manner to the longitudinal muscles in the wall of the tube foot (Smith, 1947). Protraction or extension of an individual tube foot is brought about by the contraction of its ampulla which forces fluid into the lumen of the tube foot. When the longitudinal muscles of the foot are relaxed it extends thus stretching the longitudinal muscles. Contraction of the extended longitudinal muscles in the tube foot wall shorten the foot and force fluid back into the ampulla.

Since the operation of the foot is basically a hydraulic mechanism it depends upon the maintenance of a constant fluid volume in the tube-foot-ampulla system. The maintenance of the fluid volume in the water vascular system has been considered to be the function of the madreporite and stone canal as originally suggested by Sharpey (1839). Presumably, cilia associated with the madreporite and stone canal

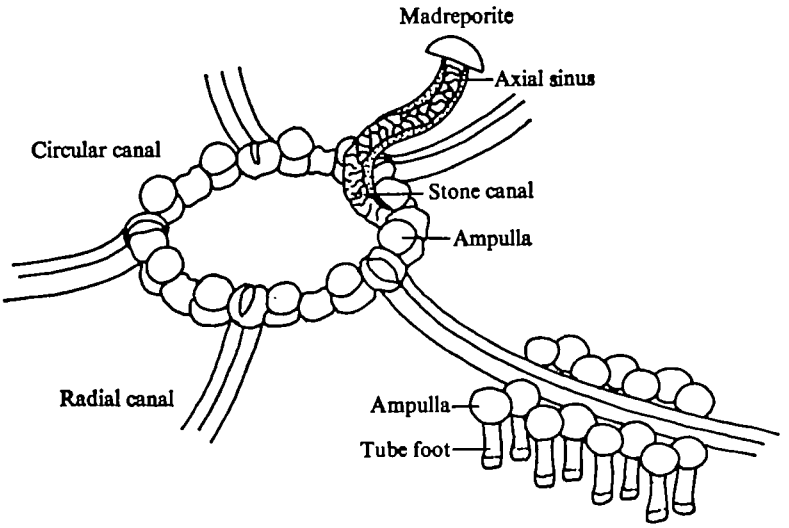

Fig. 1. Schematic representation of the water vascular system in *Asterias forbesi*.

move sea water into the canals of the water vascular system and subsequently into the tube feet by beating inwards. The constant activity of the cilia moving water into the water vascular system could compensate for water losses from the system through structural damage or possible ultrafiltration water losses across the tube foot epithelium during muscular contraction. Hyman (1955) pointed out that there was no experimental evidence in support of this now commonly held assumption in *Asterias*.

Observations concerning the ambiguity of the ciliary currents across the madreporite (Gemmill, 1914; Bamber, 1921; Budington, 1942) and elevated potassium concentrations of the ambulacral fluid (Robertson, 1949; Binyon, 1962) do not support the view that the fluid within the tube feet is simply sea water pumped across the madreporite by ciliary activity. This study was undertaken to determine how the fluid volume in the tube feet is maintained in the starfish, *Asterias forbesi*. A preliminary account of this investigation has previously been published (Prusch & Whoriskey, 1976).

MATERIALS AND METHODS

All experiments were performed with the starfish *Asterias forbesi* which were collected locally from waters off Jamestown, Rhode Island. Animals were held for up to 1 week before experimentation in artificial-sea water tanks which were aerated and maintained at 10 °C. The animals were not fed during this period.

Fluid from the tube feet lumen and perivisceral fluid was collected and analysed for ionic content (Na^+, K^+, and Cl^-) and freezing-point depression. Fluid from the tube foot was obtained by tying off a projected tube foot close to its base with silk thread. The foot was then cut off, rinsed quickly in distilled water and blotted dry on tissue paper. It was then cut open and the contents taken up in a micropipette. Perivisceral fluid was obtained by inserting a syringe through the ambulacral groove

into the perivisceral coelom and withdrawing a small amount of fluid. Na^+ and K^+ concentrations in the tube foot and perivisceral fluid were determined by flame-photometry while Cl^- was measured using a modified micro-potentiometric titration technique (Ramsay *et al.* 1955). Freezing-point depression of the various fluids was determined with a Clifton nanolitre osmometer (Clifton Technical Physics).

The ionic regulatory capabilities of intact starfish were investigated by equilibrating animals in artificial sea water with either altered K^+ or Na^+ concentrations. In the case of Na^+, the osmolality and ionic strength of the external sea water medium was maintained constant by substituting choline for Na^+. At the end of the 24 h equilibration period, samples of perivisceral and tube foot fluid were collected and analysed for the appropriate cation concentration.

To investigate the secretory capabilities of the tube foot epithelium, sac preparations were made from individual tube feet. A tube foot was severed at its base from an intact animal and flushed out with fresh sea water by sliding the open end of the isolated foot over the end of a blunt and smooth syringe needle. The isolated tube foot was then tied off with silk thread thus making a sac which contained approximately 10 to 15 μl of sea water, care being taken not to unduly expand the tube foot. The isolated tube foot sac was then allowed to equilibrate in sea water for a predetermined period. Since both sides of the tube foot now contain sea water, the concentration of all ions is initially identical on both sides of the tube foot epithelium. At the end of the equilibration period, fluid from the sac was obtained as described above and analysed for K^+. An identical series of experiments were performed except that CN^- (1×10^{-4} M) was present in the external sea water.

Unidirectional K^+ fluxes were measured across the isolated tube foot epithelium using a technique which had previously been developed for an insect-sac system (Prusch & Benos, 1976). Briefly, the open end of an isolated tube foot was slid over the end of a 19-gauge syringe needle mounted vertically in a plastic container (10 c.c.). A gap, 2 mm long, had previously been filed half way through the shaft of the needle. When the open end of the tube foot was over the end of the needle, the terminal disc of the tube foot was cut off, thus creating a cylindrical piece of tissue. The tube foot was then slid along the shaft of the needle with a pair of fine forceps until it covered the gap in the needle shaft and tied in place with silk thread. A piece of polyethylene tubing was then used to connect the end of the syringe needle to the outside of the chamber. Perfusion of the tube foot epithelium was accomplished with a Harvard syringe pump with the external bath being constantly stirred.

Unidirectional K^+ influx was measured by adding K^{42} (New England Nuclear) to the outside medium (10 μC ml^{-1}) and monitoring its rate of appearance in the perfusate. The total volume of perfusate (13·6 μl min^{-1}) was collected in metal planchettes, dried, and counted in a gas-flow counter (Nuclear-Chicago). Unidirectional K^+ efflux was measured by adding K^{42} (10 μC ml^{-1}) to the perfusion medium and monitoring its rate of appearance in the outside bath. Aliquots of the external medium (100 μl) were removed from the external bath at various time intervals and counted as described previously. Constant volume was maintained by replacing each aliquot taken with an equal volume of fresh medium. Unidirectional K^+ fluxes were calculated as described previously (Prusch, 1974).

Exchange of material in the outside sea water with the water vascular system

Table 1

Ion concentration (mM/l)	Sea water	Tube feet fluid	Perivisceral fluid
Na^+	469 ± 2·9 (17)	471·4 ± 2·6 (17)	470 ± 3·1 (17)
K^+	9·5 ± 0·8 (21)	17·3 ± 1·8 (21)	10·1 ± 1·4 (21)
Cl^-	543·3 ± 2·6 (19)	551·7 ± 3·1 (19)	541·7 ± 2·3 (19)
Total osmolality (m-osmoles/kg)	1095 ± 3·3 (25)	1117 ± 3·8 (25)	1097 ± 3·9 (25)

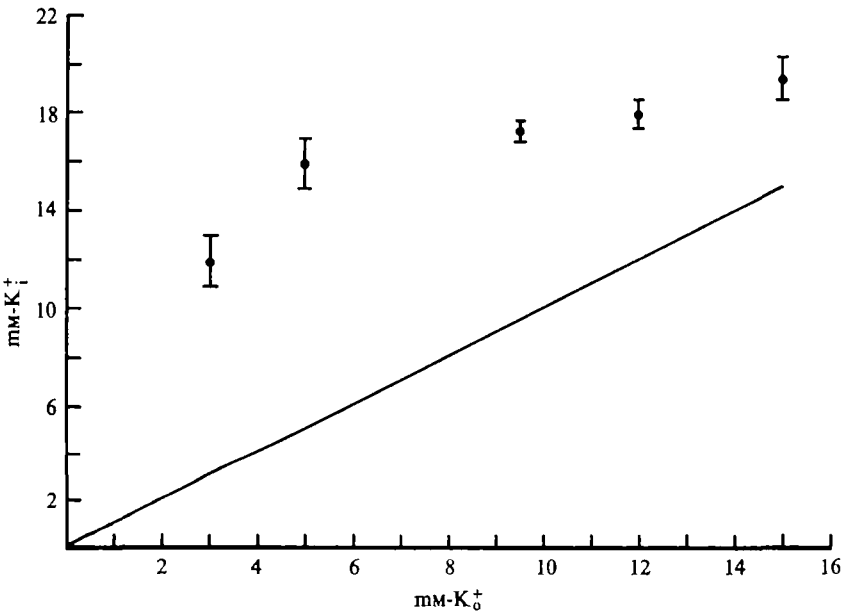

Fig. 2. Tube foot K^+ concentration in intact starfish as a function of changes in external sea water K^+. Animals were equilibrated in sea water with various K^+ concentrations for 24 h. The solid line represents the K^+ 'iso-concentration' line.

across the madreporite was investigated by equilibrating intact starfish for 24 h in sea water with [^{14}C]polyethylene glycol (New England Nuclear; M.W. = 4000). At the end of the equilibration period, samples of tube foot fluid were collected, placed into scintillation vials with 15 ml Aquasol (New England Nuclear) and counted in a liquid scintillation counter. Results are expressed as the mean ± S.E.M. and number of determinations.

RESULTS

The ionic concentration and osmolality of the external medium, perivisceral fluid and tube foot fluid (ambulacral fluid) of *Asterias forbesi* is summarized in Table 1. The most striking differences between the tube foot fluid and the external environment are in the K^+ content and total osmolality. The K^+ concentration in the tube foot is 17·3 ± 1·8 mM as compared with 9·5 ± 0·8 mM in sea water. These values are consistent with those found in *Marthasterias glacialis* (Robertson, 1949) and in *Asterias rubens* (Binyon, 1962). The Cl^- concentration of the tube foot fluid (551·7 ± 3·1)

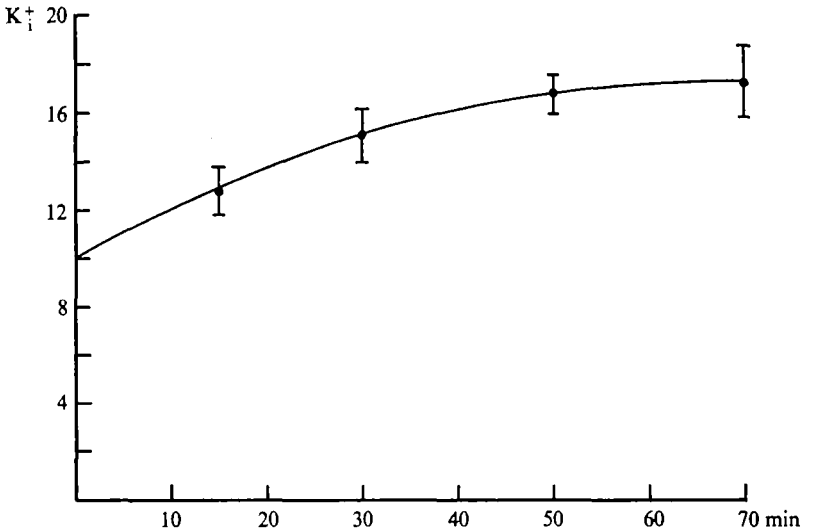

Fig. 3. K^+ secretion by isolated tube feet-sacs with time. Initially, sea water is present on both sides of the tube foot-sac.

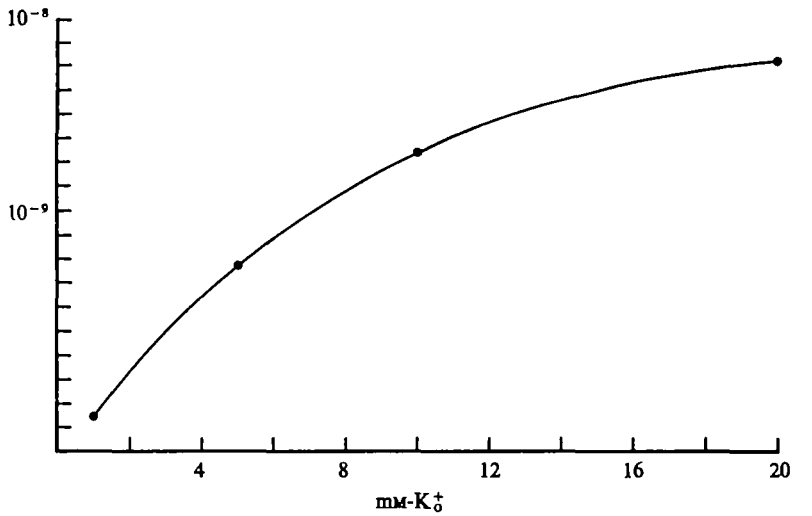

Fig. 4. Unidirectional K^+ influx across the isolated tube foot epithelium as a function of the external K^+ concentration.

is also higher than that in the external medium (543·3 ± 2·6 mM). The tube foot fluid is hyperosmotic by approximately 22 m-osmoles, as compared to sea water. The perivisceral fluid, on the other hand, was of similar ionic and osmotic concentration compared to sea water.

The K^+ concentration of the tube foot fluid is regulated over a relatively wide concentration range in the external medium (Fig. 2). Perivisceral K^+ and Na^+ levels and the Na^+ concentration of the tube foot fluid are essentially the same as in the surrounding medium. These observations are similar to those made in *Asterias rubens* (Binyon, 1962).

The hyperosmicity and K^+ content of the fluid in the tube feet suggests that it is not simply sea water pumped across the madreporite. The increased K^+ content of the tube feet fluid could conceivably be brought about by active K^+ secretion by the walls of the tube feet, as originally suggested by Robertson (1949). The K^+ content of isolated sac preparations increased from an initial value of 9·5 mM to 16·8 ± 1·9 (11) mM after 60 min (Fig. 3). This secretion of K^+ by the isolated tube foot epithelium could be inhibited by the presence of CN^- (1×10^{-4} M) in the external medium. Experiments with K^{42} showed the unidirectional K^+ influx to be $4{\cdot}2 \times 10^{-9}$ moles.cm^{-2}.min^{-1} and efflux was $3{\cdot}4 \times 10^{-9}$ moles.cm^{-2}.min^{-1}. Furthermore, unidirectional K^+ influx demonstrates saturation kinetics with increasing external K^+ (Fig. 4). No appreciable transepithelial potential (i.e. 0·1 mV) existed across the isolated epithelium.

Fluid flow across the madreporite, from the outside sea water into the more distal portions of the water vascular system and tube feet, was investigated by equilibrating intact starfish for 24 h in sea water with [^{14}C]polyethylene glycol (M.W. = 4000). At the end of the equilibration period, no trace of label was found in the tube feet fluid. Since the polyethylene glycol molecule is quite large, it could not simply diffuse across the tube foot epithelium and into the lumen, but would have to enter the tube feet through the canal structures of the water vascular system (Fig. 1). Since no polyethylene glycol was found in the tube feet lumen, it is doubtful that the fluid present in the lumen of the tube feet is outside sea water which was moved there by ciliary activity through the water vascular system.

DISCUSSION

The water vascular system of asteroids consists of a series of interconnecting canals (Fig. 1). The apparent connection of the water vascular system with the outside sea water occurs through the madreporite, which is located interradially on the aboral surface of the animal. Water currents have been observed to occur in both directions across the porous madreporite (Gemmill, 1914; Bamber, 1921). The madreporite is connected to the circumoral circular canal by the calcified stone canal, with radial canals running from the circumoral ring along the length of each arm. In *Asterias forbesi*, four rows of tube feet project from the oral surface of each arm with each tube foot connected to the radial canal through a lateral canal. A muscular valve directed so that water can only enter the tube foot-ampulla system from the radial canal (Smith, 1946) isolates each tube foot from the rest of the water vascular system. The major function classically assigned to the water vascular system was the provision of fluid for the operation of the tube feet.

The tube feet in asteroids function primarily in locomotion. The circumferential muscles of the ampulla act antagonistically to the longitudinal muscles of the foot through the fluid contained within the tube foot-ampulla unit. When the muscles of the ampulla contract, fluid is expelled into the foot causing the foot to project further and stretching the longitudinal muscles of the foot. The foot is prevented from simply swelling or increasing greatly in diameter by the presence of circular connective tissue (Smith, 1946). Contraction of the longitudinal muscles of the projected foot shortens the foot and expands the ampulla by forcing fluid out of the

foot back into the ampulla while bending of an individual foot is brought about by the contraction of the longitudinal muscles on only one side of the foot.

The tube feet of the starfish, acting together, bring about directed movement of the animal. The tube feet may either pull the animal along the substratum or the feet may act as levers with the animal 'walking' (Kerkut, 1953). The adhesion of the feet to the substratum is brought about by mechanical action and the secretion of a mucus-like substance by secretory cells at the base of the tube feet which may act as a glue (Chaet & Philpott, 1964). In addition to locomotion, tube feet function in feeding, either for holding and tearing apart prey (Christensen, 1957) or in the direct uptake of organic nutrient material directly across the tube feet epithelium (Furguson, 1967). Tube feet may also function as a respiratory surface in some echinoderms (Meyer, 1935; Farmanfarmaian, 1966).

To enable the tube feet to function, the fluid volume in the tube foot-ampulla system must be constant since the unit functions as a closed hydrostatic unit. Although it has been suggested that ciliary pumping of sea water through the water vascular system maintains the fluid volume of the tube feet, there has been no experimental evidence in support of this view. In fact, there are several observations which would appear not to support this assumption. For example, Smith (1946) found the volume of the fluid in the water vascular system to only 1 to 2% of the total capacity of the tube feet-ampulla systems and suggested that this was an insufficient amount of fluid to supply all the tube feet of the animal. In the urchin *Echinus esculentus*, Fechter (1965) found no flow of fluid across the madreporite under conditions of equal hydrostatic pressure across the system. Furguson (1967) investigated the uptake of [^{14}C]labelled amino acids in starfish and found the radioactivity to be confined to the externally exposed regions of the madreporite (among other external areas). There was no significant radioactivity in the stone canal. If the external sea water was being pumped across the madreporite into the water vascular system, then in this case labelled amino acids should have been found in the tissues associated with the stone canal. The presence of elevated K^+ levels in the tube feet fluid (Robertson, 1949; Binyon, 1962) also indicates that the fluid in the tube feet is not simply sea water pumped into the water vascular system. Further evidence for the absence of bulk flow of sea water across the madreporite and into the tube feet was shown in this study by the lack of labelled polyethylene glycol penetration into the tube feet lumen from the external medium.

If there is, apparently, no bulk flow of sea water across the madreporite into the tube feet lumen, the question arises as to how the fluid volume in the tube feet is maintained. It has been shown in other studies (Binyon, 1976) that an increase in hydrostatic pressure across the tube feet epithelium (such as would arise by muscular contractions in the system) brings about a large increase in the efflux of water from the tube foot lumen. For this hydrostatic unit to function efficiently, this water loss must somehow be made good.

It is conceivable that in *Asterias forbesi* the maintenance of the fluid volume in the tube feet systems is brought about by the secretion of K^+ ions by the tube feet epithelium into the lumen of each tube foot. The measured K^+ and Cl^- concentrations, and total osmolality, are higher in the tube foot fluid of intact animals than in the outside sea water (Table 1). This elevation of K^+ concentration is probably brought

about by active secretion by the tube foot epithelium and possibly that of the ampulla. Active K^+ secretion is indicated from the increase in K^+ in isolated tube foot-sacs with time (Fig. 3) and from unidirectional K^+ measurements across the isolated epithelium. The secretion of K^+ can be inhibited by the presence of external CN^- (1×10^{-4} M) and unidirectional K^+ influx demonstrates saturation kinetics (Fig. 4). In addition, K^+ levels in the ambulacral fluid are regulated at a nearly constant level over relatively wide changes in external K^+. Apparently, the secretion of K^+ by the tube foot epithelium is electroneutral with Cl^- following passively.

The direct secretion of K^+ by the tube foot epithelium in the observed absence of bulk water flow across the madreporite into the water vascular system, could account for the maintenance of the fluid volume in the tube feet. Secretion of K^+, with Cl^- following, into the lumen of the tube foot (which is closed off from the rest of the water vascular system by a muscular valve) could elevate the osmolality of the tube foot fluid by the observed 20 m-osmoles (Table 1). This elevation of the total solute concentration, which produces tube foot fluid that is slightly hyperosmotic with respect to the outside medium, could provide the driving force for the maintenance of the necessary fluid volume of the tube feet.

If the fluid volume in the tube feet is maintained by solute secretion, then another question is raised, namely: what is the function of the madreporite and tubular portions of the water vascular system in *Asterias*? In the urchin *Echinus esculentus*, where no fluid movement has been found to occur across the madreporite, Fechter (1965) suggested that the opening of the ambulacral system through the madreporite serves to eliminate any pressure differences across the system. It is possible that the madreporite in *Asterias* also functions in this way. It is, in addition, conceivable that the water vascular system in asteroids could have a sensory function. For example, *Asterias rubens* responds positively towards bivalve prey (Castilla, 1972). This chemoreceptive response could, in part, be a function of some structure associated with the water vascular system.

If the water vascular system in the asteroids primitively supplied fluid for the operation of the tube feet, it may still have this function periodically in *Asterias*. Occasionally, when great demands are placed on the tube feet (such as during prolonged effort to open a bivalve prey) the loss of water could be very great. Water could then be pumped quickly from the outside medium through the water vascular system and into the tube feet. The water vascular system may thus have primitively had the primary function of maintaining the fluid volume of the tube feet. In present-day asteroids, however, the water vascular system only fulfils this function during periods of stress.

The advantages of maintaining fluid in the tube feet by solute secretion by the epithelium rather than by water pumping through the water vascular system are unknown. The tube feet system in echinoderms has apparently evolved from the pattern observed in Crinoids, in which the feet are protracted by hydraulic pressure created in the water vascular canal, to that observed in the asteroids, in which each tube foot can operate independently from the rest of the water vasculary system. According to Nichols (1966), one of the advantages of the ampullary method is that the canals of the water vascular system are permanent through-channels for the

passage of fluid. Maintenance of fluid volume in the tube feet by solute secretion could free the water vascular system from having to fulfil this function in asteroids, except perhaps as an emergency device, thus leaving it free to assume other functions.

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