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RATE OF WATER ADMISSION THROUGH THE MADREPORITE OF A STARFISH

BY JOHN C. FERGUSON

Department of Biology, Eckerd College, Box 12560, St Petersburg, FL 33712, USA

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Summary

Time course studies employing the fluorescent, high molecular weight tracer, fluorescein isothiocyanate dextran, confirm that there is a steady entry of sea water through the madreporite of the starfish, *Echinaster graminicola*, even under constant environmental conditions. This entry serves to supply replacement fluid to both the water vascular system and the perivisceral coelom, with more going to the latter than the former (10·67 *vs* 6·35 μl h^{-1} for a 7·25 g animal). Since nearly 2·4 days may be required for an animal to take up 1 ml of sea water through the madreporite, the rate of entry is too low to be easily seen in direct observation, or to have immediate physiological consequences if prevented. Nevertheless, the rate is probably sufficient to make a substantial contribution to fluid volume regulation, functioning along with osmolyte transport and other processes that have been emphasized in recent literature.

Introduction

Echinoderms are unique among major groups of animals in that they have no recognizable excretory organs to assist them in maintaining fluid and osmotic balance, and they do not possess impervious integuments to protect them from the vagaries of the external environment. Unlike many other forms, they presumably do not drink and excrete water or ions to maintain fluid homeostasis. Most sources describe them as being simply 'isosmotic' and dismiss the question of how they regulate fluid volume. This lack of consideration is especially surprising considering that echinoderms are completely dependent on hydraulic mechanisms to drive their appendages, and a number of species are soft-bodied, relying partially on internal turgor to support their shape or at least to inflate their respiratory papulae. Clearly, even in stable environments, most echinoderms face continual losses of fluid that must be balanced.

The most obvious way that this might be accomplished is by admitting sea water through the madreporite into the water vascular system, a complex of vessels peculiar to echinoderms. However, until recently there has been little evidence for this, and several workers have questioned the importance of these organs in

Key words: *Echinodermata*, Asteroidea, *Echinaster*, water-vascular system, osmoregulation.

normal fluid volume regulation. The principal challenge has come from Binyon (1962, 1964, 1966, 1976*a*,*b*, 1980, 1984). He observed that severed starfish arms maintained tube feet activity for many hours after they had been isolated from the madreporite and stone canal. In repeated attempts, he and others could not see fluid entering the madreporite, and dye particles were always swept away from this structure. Finally, following earlier suggestions by Robertson (1949), Binyon detected a significant elevation of potassium ion concentration in ambulacral fluid, showing that the fluid had a composition different from sea water, and that it was likely to draw water osmotically from the environment. Prusch & Whoriskey (1976) and Prusch (1977) confirmed elevated levels of K^+ (and Cl^-) in ambulacral fluid, and demonstrated active transport of K^+ in the tube foot wall. Prusch (1977) also stated that he was unable to detect movement of labeled polyethylene glycol into the madreporite.

In response to these earlier studies, current literature has begun to discount the importance of the madreporite mechanism in tube foot functioning, pointing instead to ionic transport as the process of primary concern. In this view, the madreporite is seen as perhaps a sense organ, a relief valve or a vestigial anomaly. At most, some think it may function intermittently as a supplemental pump in periods of osmotic stress.

Recently, I reported a flaw in this re-assessment (Ferguson, 1988). If the osmolarity of the ambulacral fluid is elevated (as it appears to be), then it would tend to draw water into the water vascular system, not just by permeation through the thick walls of the tube feet but also through the very thin ampullary membranes. This should lead to fluid depletion from the perivisceral coelomic cavity, which has no obvious way to regain it. Thus, concern has been focused too narrowly on how the water vascular system might maintain homeostasis and not on how the animal itself does. The former must be considered in the context of the latter.

Ferguson (1988) showed that, over a period of 18–20 h, substantial obstruction of the madreporite of a starfish (*Echinaster*) could lead to statistically significant losses of mass (fluid). Further, after a similar period of exposure, fluorescently labeled dextran tracer with a high molecular mass had entered both the ambulacral and the perivisceral coelomic fluids of experimental animals. This entry was significantly reduced (70 and 83·5%, respectively) by madreporite obstruction. (Completely sealing the soft tissue of the madreporite for long periods did not prove to be feasible.) Additional, as yet unpublished, tracer experiments produced similar results. Aerially dehydrated animals take up water and tracer more rapidly than normal animals, and this is significantly inhibited by madreporite obstruction. Madreporite obstruction also causes interference during adjustments to both hyper- and hypo-osmotic stresses.

From these results, Ferguson (1988) concluded that the madreporite must admit sea water and play an important role in starfish fluid volume regulation, along with mechanisms of ionic transport, digestive system pumping and perhaps other factors. The present study provides the first quantitative estimate of actual water

inflow through the madreporite of a starfish under 'normal', stable conditions. In addition, the tracer methods used show the relative importance of this inflow to the water vascular system compared with the major body compartment.

Materials and methods

Specimens of *Echinaster graminicola* Campbell & Turner, 1984, were collected from tidal grass flats in Tampa Bay in late summer after all spawning activity had been completed. They were kept in the laboratory for at least 2 days under stable conditions, in containers of sea water obtained with them, and carefully maintained at the original environmental temperature and salinity. Mean mass of the specimens used was 7·25 g, with individuals ranging from about 3 to 12 g.

For the experiments, pairs of animals were placed in dishes containing 200 ml of filtered sea water (from their aquaria) and about 20 mg of dissolved fluorescent tracer, fluorescein isothiocyanate dextran (FID) (Sigma Chemical) with a relative molecular mass (M_r) of 70000. FID seems to be completely innocuous to the animals and does not pass through membranes. This has been verified by the results of experiments (Ferguson, 1988) and by observations that it was unable to penetrate dialysis bags containing sea water.

After appropriate periods of incubation in the labeled media, animals were removed, rinsed three times in sea water, and weighed. Their perivisceral coelomic fluid (PCF) was then drained into a vial by snipping off the tip of one arm. Two other arms (the bivium) were removed from the body for duplicate measurements of ambulacral fluid (AF) levels. The top half of each of these arms was removed, along with the internal organs, and the lower half thoroughly rinsed in a stream of filtered sea water. After the arm had been drained on a towel, its inside was carefully blotted dry (twice) with pieces of glass fiber filter paper (Whatman, GF/A). An incision was then made through the ampullae with a scalpel and about 10 μl of ambulacral fluid drawn on to a preweighed (in a fluorimeter tube) piece of glass fiber paper. This was immediately dropped back into a fluorimeter tube. After quickly weighing the tube and paper (to minimize evaporation), 4 ml of phosphate buffer (17·9 g l^{-1} $Na_2HPO_4 \cdot 7H_2O$) was added and the tube vortexed several times. The paper was then removed and the tube set aside (to settle turbidities) for 1 h before reading.

Fluorescence in the samples was measured on a Turner 110 fluorimeter using 2A and 47B primary and 2A-12 secondary filters. Readings were adjusted to the equivalent of a 10 μl sample, based on the ratio of mass differences. Media and PCF levels were also measured in duplicate as 10 μl samples in 4 ml of buffer. The drawn perivisceral coelomic fluid was allowed to settle for 5–10 min before the samples were taken. All media measurements were normalized to 100 (10 'units' per μl) and concentrations in AF and PCF expressed relative to this standard.

An estimate of the volume of ambulacral fluid was made by cutting out photographs of arm cross-sections, weighing them, and comparing them with the mass of cut-outs of the ambulacral spaces. Values between 3 and 5 % of the body

volume were obtained, and 4% was taken as the norm. For an approximate measure of the perivisceral coelomic volume (which may vary according to the state of the animals), specimens were weighed and their fluid thoroughly drained through severed arm-tips, after which they were reweighed. A normative value of 25% of the body mass was taken from these data.

Results

Levels of FID increased in both the AF and PCF throughout the course of the experiments, but were always higher in AF (Figs 1, 2). Variations occurred in the

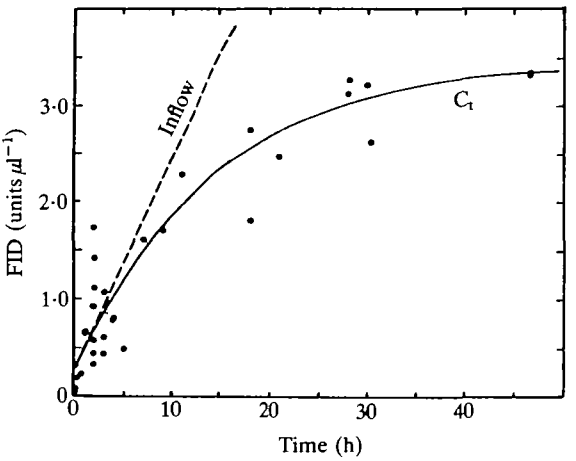

Fig. 1. Build-up of FID in ambulacral fluid. Line C_t shows a concentration-dependent loss. The upper line (Inflow) represents the increase in concentration without such loss.

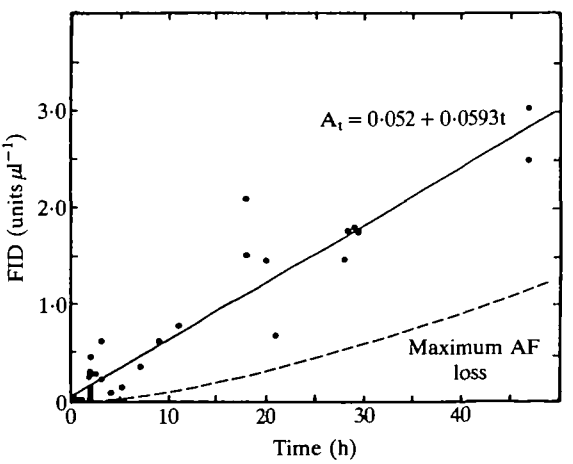

Fig. 2. Accumulation of FID in perivisceral coelomic fluid. Data are fitted ($r = 0{\cdot}95$) to the least-squares line (A_t). Losses from ambulacral fluid could not account for more than that shown by lower line.

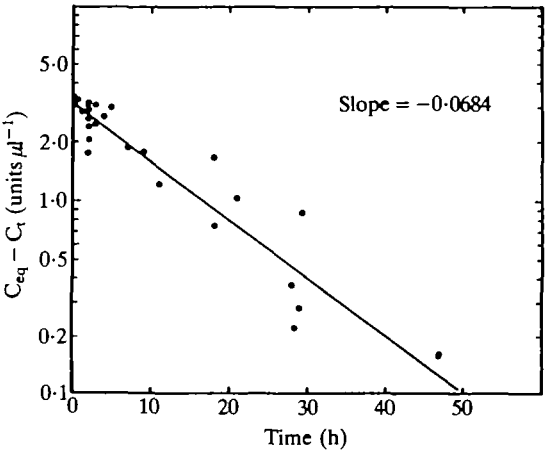

Fig. 3. Ambulacral fluid; equilibrium level of FID minus measured concentration. The line is from the least-squares fit ($r = 0{\cdot}96$) of log-transformed data.

responses of individuals, but clear overall patterns could be discerned. The rate of build-up of FID in the AF decreased with time, leveling off at a concentration of about one-third of that of the medium, whereas the build-up in the PCF continued almost uniformly throughout the 2 days of observation.

Ambulacral fluid

Assuming a leveling off (C_{eq}) of the AF concentration at 3·5 units μl^{-1}, a semilogarithmic plot of this minus the observed level (C_t) at each time (t) produces a straight line (Fig. 3). By logarithmic transformation and least-squares regression, this line can be defined as:

$$\ln(C_{eq} - C_t) = 1{\cdot}162 - 0{\cdot}0684t,$$

with a correlation coefficient (r) of 0·96 ($N = 31$).

This result would fit a model with a steady rate of inflow of tracer through the madreporite (I units h^{-1}) into the AF compartment (volume V μl) and a concentration-dependent loss from this compartment (LC units h^{-1}). This type of dynamic system is well known and has been discussed by Simon (1972). He showed the change in a quantity (Q) as:

$$\Delta Q = \Delta t(I - LC),$$

or

$$dC/dt = (I - LC)/V,$$

which, with Laplace transformation, becomes:

$$C_t = (I/L)(1 - e^{-(L/V)t}).$$

Since at long time intervals the exponential term diminishes, the steady-state condition may be represented as:

$$C_{eq} = I/L .$$

Thus:

$$C_t = C_{eq}(1-e^{-(L/V)t}) .$$

Applied to the present data, the exponent $-(L/V)$ can be taken from the least-squares slope:

$$L/V = -0{\cdot}0684 .$$

A difference was observed between the calculated y-intercept ($C_{eq}-C_{t=0}$) and the estimated equilibrium level (C_{eq}). This difference, considered to be 'background' interference (B), was equal to 0·304. In large measure it probably reflected residual fluorescence in the samples as well as variation between the animals and their true equilibrium levels.

Incorporating these variables, the data presented in Fig. 1 can be modeled as:

$$(C_{eq}-B)(1-e^{-(L/V)t})+B$$

or:

$$C_t = (3{\cdot}5-0{\cdot}304)(1-e^{-0{\cdot}0684t})+0{\cdot}304 .$$

This curve is plotted in Fig. 1.

The rate of tracer inflow (I units μl $AF^{-1}\,h^{-1}$) through the madreporite into the AF compartment can be determined from:

$$I = (C_{eq}-B)L;$$

where $L = 0{\cdot}0684V$,

$$I = 0{\cdot}219V .$$

This inflow (per μl AF) is also shown in Fig. 1.

The final determination of I for an animal requires knowledge of the volume of the AF compartment. This was estimated to be 4 % of the body volume which, for an average 7·25 g specimen, can be taken as 290 μl. Thus,

$$I = (0{\cdot}219)(290) = 63{\cdot}5 \text{ FID units } h^{-1} .$$

Since the medium concentration was 10 FID units μl^{-1}, inflow of sea water through the madreporite to supply ambulacral fluid is 6·35 $\mu l\,h^{-1}$.

At this rate, 2·2 % of the AF would be replaced each hour, 45·7 h would be required to replace all the 290 μl of the water vascular system, and 6·57 days to draw in 1 ml of sea water for this replacement.

Perivisceral coelomic fluid

In contrast to the AF levels, build-up of the tracer in the PCF was a slower but steadier process. It showed no sign of a concentration-dependent loss (Fig. 2). A regression line indicated an accumulation rate (A) of 0·0593 FID units μl $PCF^{-1}\,h^{-1}$, with a y-intercept of 0·052. The correlation coefficient (r) of the fit was 0·95.

It is possible that the tracer accumulating in the PCF represents the loss observed from the AF. Were this to be the case, the accumulated concentration in the PCF would be given by the total quantity admitted to the ambulacral system minus the amount retained there, divided by the volume of the PCF:

$$A_t = \frac{(It)-(C_t-B)V_{af}}{V_{pcf}},$$

where the terms are as above, except for the distinction between the volume of AF (V_{af}) and the volume of PCF (V_{pcf}). The latter was estimated to be about 25 % of the body volume or, for an average 7·25 g animal, 1800 μl.

Fig. 2 shows the maximum amount of tracer that could be accounted for in the PCF had it all come from the AF. This line does not fit the data. The calculated amounts consistently represented less than half the material observed in the PCF, and the shape of the slope showed little relationship to the observed data.

If, however, the input of FID into the PCF was from sea water that had entered the madreporite, but was diverted to the PCF before appearing in the ampullae (where the AF samples were taken), this input would correspond to a rate of entry of 10·67 μl of sea water per hour. Thus, for the conditions studied, the maximum rate of entry of water through the madreporite to supply separately both the AF and the PCF would be 17·02 μl h^{-1}. It should take about 2·4 days to gain 1 ml, and about 0·59 % of the PCF volume would be replaced each hour by madreporite water.

Discussion

Although the rates given here should only be considered as approximations, applying to the species and specific experimental conditions, they seem reasonable in view of other observations. For the first time they provide a clear quantitative picture of the functioning of the madreporite system in an echinoderm, and help to explain some of the perplexing properties of these animals. The data show that sea water routinely enters the madreporite, and it does this not as a spasmodic process, or one limited to periods of osmotic stress, but rather as a steady contribution to the turgor of both the water vascular system and the other coelomic spaces. The rate of entry of sea water through the madreporite is too low to be easily seen by direct visual methods. Since it represents only a small percentage of the volume of the water vascular system per hour, and osmotic mechanism might also be involved, it should not be surprising that tube feet activity and other functions can continue for long periods after input has been shut down, such as after an arm has become severed from the disc. Why Prusch (1977) failed to detect entry of labeled polyethylene glycol into the ambulacral fluid of *Asterias* is uncertain, but it may relate to the slowness of the process.

The extent to which madreporitic inflow actually satisfies the normal needs of the animal cannot be specified. The observed rates are certainly high enough to make a major contribution, but the demands of the organism are very difficult to

evaluate. Binyon (1980, 1984), studying the much larger species *Asterias rubens*, estimated permeability losses which, when scaled to *Echinaster*, are 1–2 orders of magnitude higher than the measured uptakes observed here. However, he used pressure assumptions which are more than an order of magnitude higher than I would make for *Echinaster*, and this, along with other dissimilarities of scale and species type, limit pertinent comparison.

Although it is possible that the measured inflow of sea water through the madreporite could completely satisfy the fluid needs of the animals, it is more probable that it is only one of several factors that contribute to fluid volume maintenance. As noted earlier, workers have published studies in support of a K^+ osmotic pump process in the tube feet (Prusch & Whoriskey, 1976; Prusch, 1977). Ferguson (1988) showed elevated K^+ levels in *Echinaster* PCF, and a slight positive osmotic pressure difference between the PCF and the external environment, implying that *Echinaster* is not truly isosmotic. Furthermore, fluid movements through the gut were also implicated in volume regulation. Total fluid balance, thus, probably reflects the interaction of a number of processes. However, of these, madreporite water admission is unique in that it depends only on hydrostatic pumping, and it is not subject to the kinds of biochemical and electrochemical limitations that might be restrictive to the other mechanisms.

Of particular interest is the further evidence that madreporitic inflow is important in maintaining the volume of the PCF, as well as the AF. Ferguson (1988) showed that when the madreporite was obstructed, significantly less FID tracer could reach the perivisceral coelomic cavity, and loss of mass (fluid) was enhanced. Hence, this change does not seem to be an artefact. Indeed, in those experiments, mean loss of mass when the madreporite was obstructed was equivalent to $16{\cdot}6\,\mu l\,h^{-1}$, which compares well with the $17{\cdot}02\,\mu l\,h^{-1}$ inflow calculated in the present study. Some contribution of madreporitic fluid seems essential to balance likely losses of PCF through the ampullary membranes into the osmotically elevated AF. It has now been shown that even more water enters the madreporite to move directly into the perivisceral coelomic cavity than flows into the tube foot–ampulla portions of the water vascular system (perhaps a ratio of as high as 10·67:6·35). Because of the osmotic elevation of the AF, some of this water might ultimately move from the PCF to the AF, and by this indirect route contribute to tube foot hydraulics. Certainly, it must also help maintain general body turgor.

The results do not indicate that larger molecules, such as FID tracer, can move from the AF cavity to the PCF cavity. These substances (unlike water) should not be expected to pass through the ampullary membranes. The losses of FID observed from the AF must be attributed to a purification process operating in the water vascular system; some such process doubtless exists in any situation involving steady inflow of sea water, with all its natural constituents, into a dead-end compartment. The nature of this purification is still under study, but preliminary observations with fluorescence microscopy point to the tube feet coelomic lining cells and the coelomocytes contributing to the removal of organic

material. The lack of obvious FID loss from the PCF is more perplexing. The PCF, too, must be purified of unwanted organic material but, possibly because of its large volume compared with that of the water vascular cavity, and other factors, this purification is not obvious.

How FID reaches the PCF after its entry through the madreporite is also unclear. The existence of a direct passage is implied by this study. Reports exist (Hyman, 1955; Nichols, 1962, 1966) of an open communication in the area beneath the madreporite to the axial sinus, an extension of the perihemal coelomic system. In histological observations I have confirmed that there is such a communication between the two compartments in *Echinaster*. Nevertheless, it is hard to see how an opening above the stone canal could be very useful, although it possibly could work against the low pressures of the coelomic spaces. Even with functional passages between the central water vascular vessels and the perihemal system, sea water would still need to reach the perivisceral coelom. Extensions of the perihemal compartment probably do communicate with non-coelomic spaces in the body wall and possibly parts of the perivisceral coelom itself (Hyman, 1955; Ferguson, 1982), but these have not been well described. Clearly, more attention needs to be applied to the problem of bulk fluid movement between the several compartments.

Finally, it should be noted that there are many additional questions that should be addressed now that the importance of the madreporite system has been demonstrated. These include showing how the system responds to osmotic stress, the types of control mechanisms that might exist in madreporitic admission and stone canal pumping, the special problems of intertidal species, adaptations to scale, purification, and interactions of the different processes influencing total fluid balance and fluid balance in various body components.

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