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The Structure and Mode of Function of the Water Vascular System of a Brittlestar, *Ophioderma appressum*

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Abstract. Unlike the asteroids, which have large madreporite structures, the ophiuroid *Ophioderma appressum* possesses only two small hidden madreporite pores. Experiments with labeled amino acids, fluorescent microbeads, and surgical obstruction show that small amounts of seawater do routinely enter these pores and become distributed throughout the water vascular system; but this uptake does not seem essential. The flagellated stone canal draws its fluid from the axial sinus, to which the pores connect through a tortuous ampulla. Thus, the stone canal mainly recirculates fluid from hyponeural (perihemal) passages. That perihemal fluid is augmented by seawater from the pores. As perihemal fluid moves towards the stone canal, it passes by or through the axial organ, where nutritive materials may be removed and passed into the hemal channels. Pressure generated by the stone canal forces flow out to the oral tube feet, polian vesicles, and, through valves, eventually to the arm tube feet. Inflation of the tube feet also might occur through osmotic mechanisms, but their activity was not impeded by raising the external osmotic level with dextran. Observations indicate that negative coelomic pressures must be generated during respiratory movements, and these could lead to sufficient body fluid production (by filtration) that the need for substantive madreporitic inflows would be alleviated.

Introduction

One of the most distinctive characteristics of echinoderms is their water vascular system—a unique arrangement of fluid-filled coelomic passages and associated parts. The general form of these structures in the different echinoderm classes has been summarized by Hyman (1955) and Nichols (1966) from the works of pioneer microscopists. In most types, the water vascular passages open to the exterior through a hydropore or compound madreporite, and fluid within the system is used hydraulically to extend agile appendages, the tube feet. With their variety of functional adaptations, the tube feet are probably responsible for much of the evolutionary success of this major animal group. The water vascular system, however, is much more complex in both structure and function than is generally realized, and it presents many attributes that are puzzling and, as yet, little studied.

It is generally assumed, for example, that the madreporite admits seawater into the system, and that ciliary pumping by the stone canal connected to it forces the seawater taken in to flow out to the tube feet where it replenishes fluid losses due to their activity. That view, however, was challenged by Binyon (1964, 1966, 1976a, b, 1980, 1984), who observed that starfish tube feet remain active for long periods without access to the madreporite. He noted that ionic elevation of the water vascular fluid, particularly of potassium ion, could produce an osmotic entry of water into the structures, making madreporitic inflow unnecessary. Binyon opined that the madreporite might be a relief valve, a supplemental mechanism, or have some other unknown functions.

More recently, my own work with fluorescent molecular tracers and microbeads, and other methods, has shown that seawater does routinely enter the madreporite of all starfishes tested; but this uptake is not specifically directed at tube foot inflation. Rather, the uptake plays a more important role in keeping the spacious asteroid body filled with fluid (Ferguson, 1988, 1989, 1990a, 1992, 1994). Further, the stone canal, through connections with

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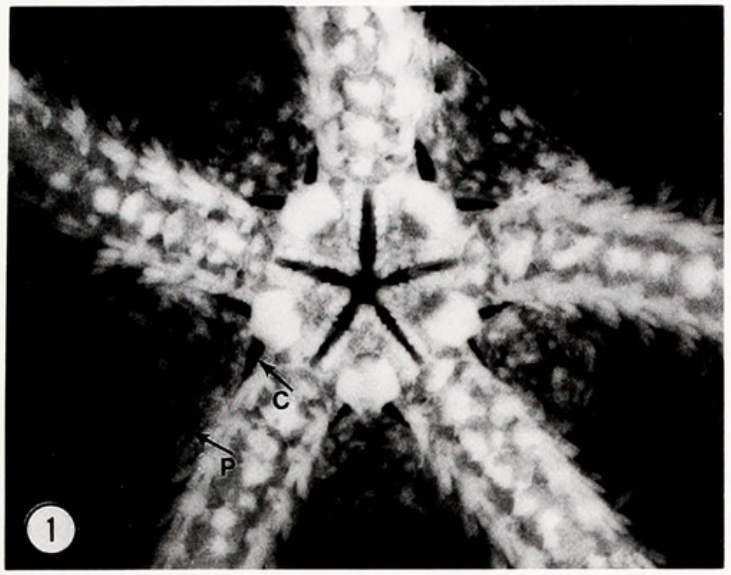


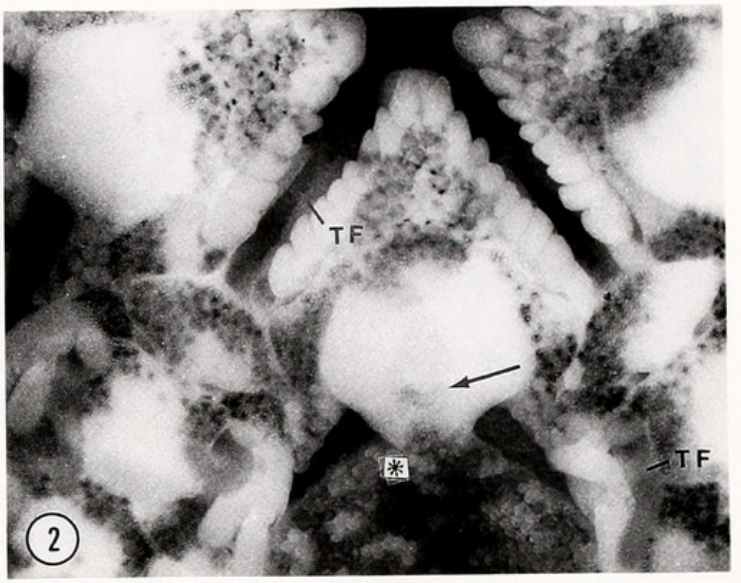


Figure 1. Oral disk of *Ophioderma appressum*. The arrows mark a pair of central (C) and peripheral (P) slits that open into one of the 10 genital bursae. Seawater is drawn from the side of the disk through the peripheral genital bursal slits and is vented by the central ones.

Figure 2. Magnified view of the oral shield overlaying the axial complex, visible as a slightly darkish area (arrow). The madreporite pores are out of sight in the crevice near the asterisk. Extended tube feet (TF) can be seen in the mouth and on the arms.

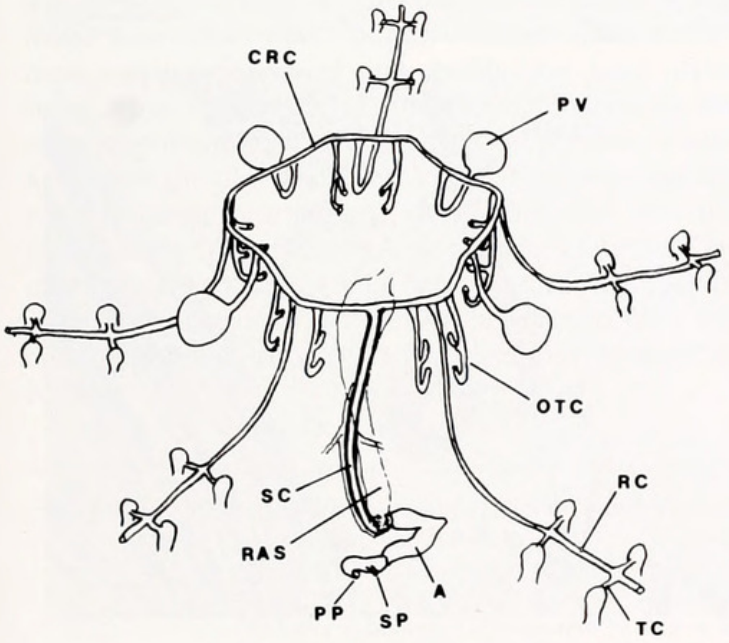


Figure 3. Organization of the water vascular system. The oral tube feet have been omitted and the arm tube feet and axial complex are only partially shown. A, ampulla; CRC, circumoral ring canal; OTC, oral tube feet canal; PP, primary madreporite pore; PV, polian vesicle; RAS, right axial sinus; RC, radial canal; SC, stone canal; SP, secondary madreporite pore; TC, transverse canal.

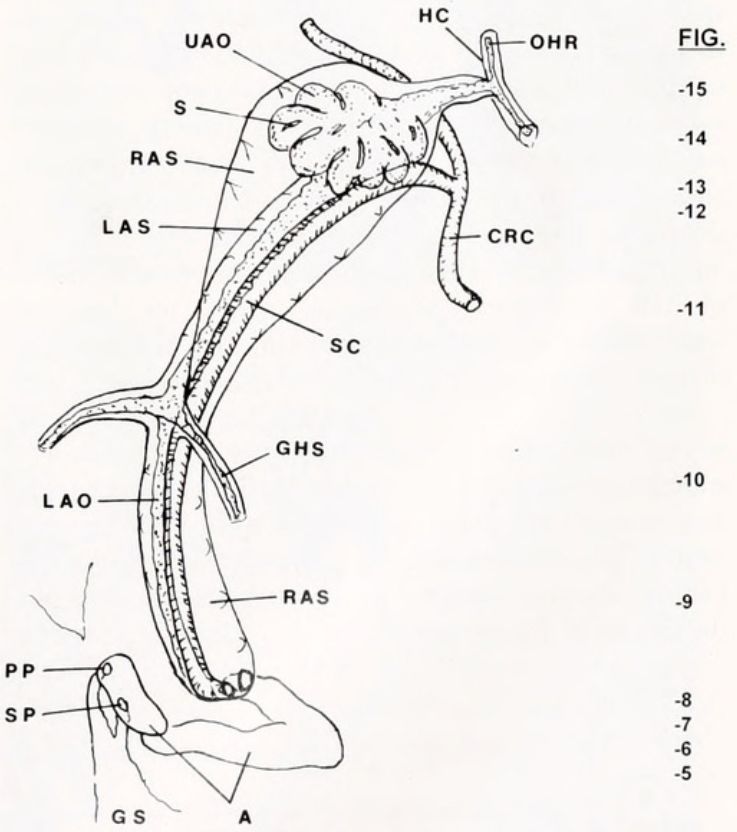
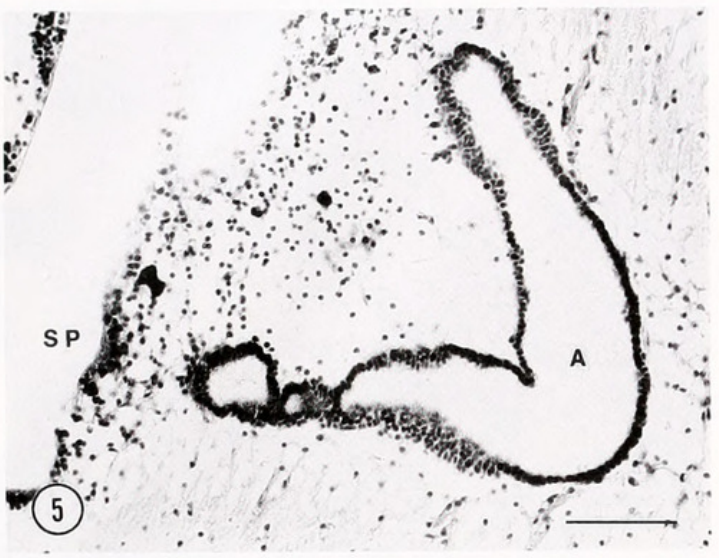
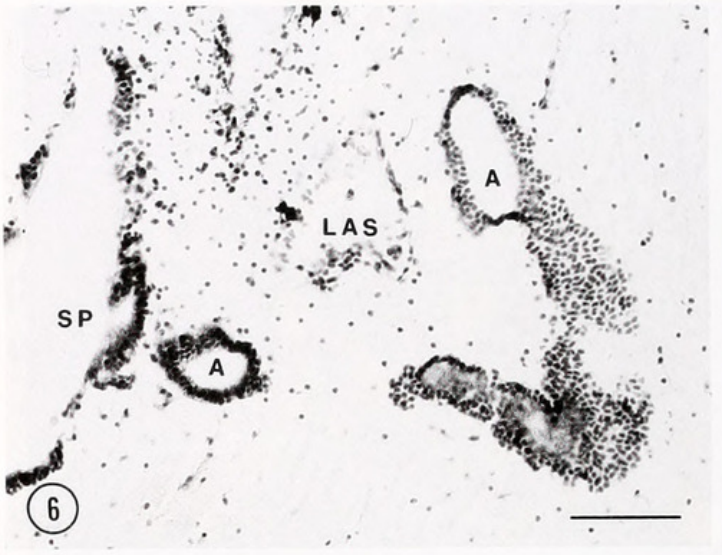
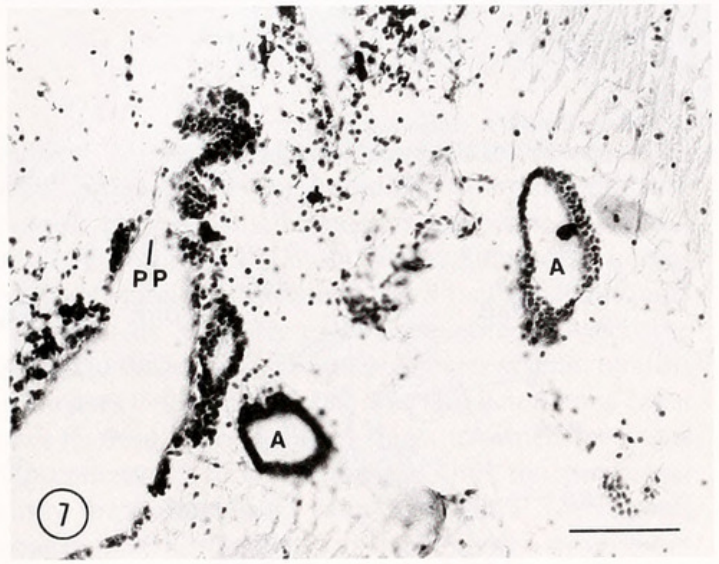
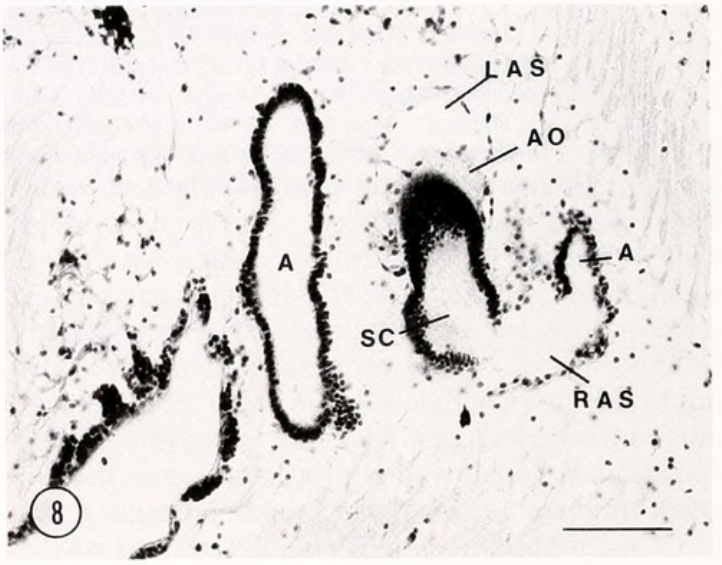


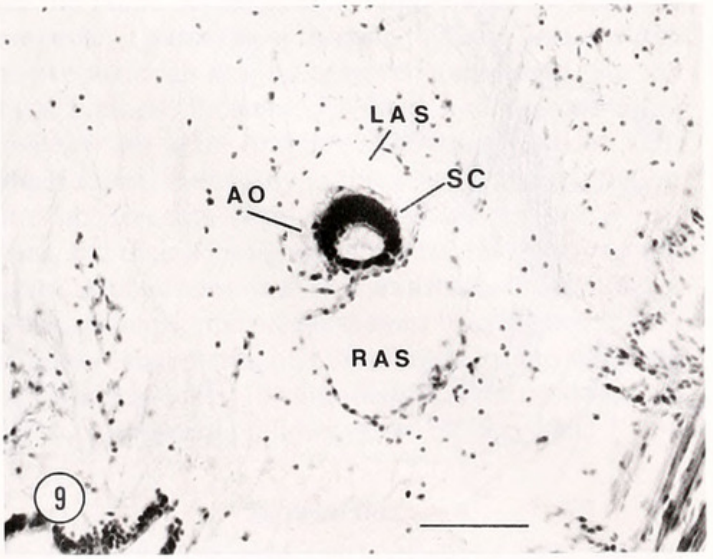
Figure 4. Detailed diagram of the madreporite-axial complex, with a scale showing the approximate level of serial sections (Figs. 5–15). A, ampulla; CRC, circumoral ring canal; GHS, genital hemal strand; GS, genital bursal slit; HC, hyponeural coelom; LAO, lower axial organ; LAS, left axial sinus; OHR, oral hemal ring; PP, primary madreporite pore; UAO, upper axial organ; RAS, right axial sinus; S, slit in axial organ connecting left and right axial sinus; SC, stone canal; SP, secondary madreporite pore.











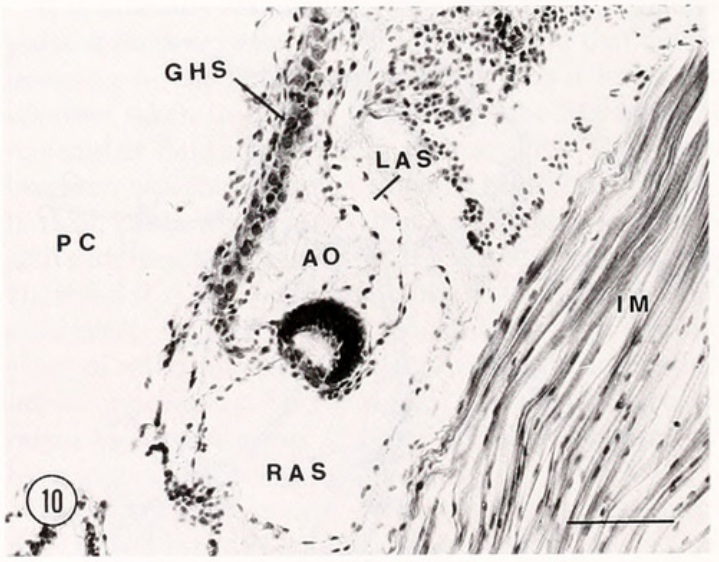


Figure 5. First of a set of serial frontal sections from the madreporite oral shield upwards. It shows part of the large, angular ampulla (A) that lies within the oral shield. The edge of the secondary madreporite pore (SP) can be seen on left, facing the cleft formed by the edge of the genital bursal slit. (All scale bars = 50 μm).

Figure 6. Section located 20 μm higher than Figure 5, showing the secondary pore opening (SP) and the lowest portion of the left axial sinus (LAS). The pore canal leads towards the site of the primary pore, away from the direction of inflow through the tortuous ampulla (A).

other parts, must maintain an internal system of circulation and filtration of the body fluids. Some starfish, such as the intertidal *Pisaster ochraceus,* cannot adequately maintain their body fluid volume without access to the madreporite, while others, such as *Pycnopodia helianthoides,* rely more heavily on osmotic uptake (Ferguson, PN100,,,L,I01992, 1994). Physical differences between species largely reflect their individual adaptations to fluid volume maintenance. *Pisaster* has a heavy, stiff, and impermeable integument, while that of *Pycnopodia* is light, flexible, and much more permeable.

Because the function of madreporite mechanism in different asteroids varies considerably, similar diversity should be expected in the other classes; indeed, differences in body form between the classes might relate to their individual approaches to solving the problems of body fluid maintenance. Although ophiuroids share many characteristics with the asteroids, they are also quite different. Here I begin a detailed examination of the structure and function of an ophiuroid water vascular system—that of *Ophioderma appressum.* Comparison of its properties with those of other echinoderms should broaden our understanding of the adaptations of these diverse organisms.

Materials and Methods

Specimens of *Ophioderma appressum* were collected from tidal grass flats in Tampa Bay and maintained in running, fresh seawater until used for experiments. This species is very similar to *Ophioderma brevispinum* (referred to in earlier work), but grows larger and possesses more equal-dimensioned oral shields (five large plates surrounding the mouth). As in other ophiodermids, the openings into its genital bursae (pairs of pouches invaginated into the disk on either side of the arm bases) are each separated into two slits—a "central" one near the mouth and a "peripheral" one on the edge of the disk. Its madreporite cannot be seen externally, and the structure of its water vascular system must be inferred from old descriptions of other ophiuroid species (*cf.* Hyman, 1955; Nichols, 1966).

Because the internal parts of the water vascular system are all small and embedded in ossicle and fibrous connective tissue, they are best studied with histological methods. To locate the site of the madreporite and examine the organization of the entire system, two small specimens (6–7 mm disk diameter) were fixed in Helly's fixative and decalcified for one week in changes of cold (4°C) 5% disodium EDTA. After paraffin embedding, serial frontal sections (from the mouth through the top of the disk) were cut at 10 μm thickness and stained in Delafield's hematoxylin, with eosin and orange G as counter stains.

The extent of seawater entry through the madreporite was evaluated in two sets of experiments. The first of these was actually an unpublished part of a former study dealing with ingestion and hemal transport by *Ophioderma* (Ferguson, 1985). A pair of brittlestars was placed for 8 h in seawater containing 100 μCi ^{14}C synthetic algal protein hydrolysate, 375 mg glycine, 990 mg glucose, and 10 mg chloramphenicol per liter. They were then fixed and decalcified in Bouin's solution, embedded in paraffin, sectioned (8 μm), and exposed for up to 6 months to Kodak AR-10 autoradiographic film. In the second set of experiments, fluorescent microbeads were employed. These had been used to study madreporite function of the starfish *Leptasterias hexactis* (see Ferguson, 1990a). Two brittlestars were placed in a dish containing about 100 ml of seawater along with about 0.5 ml of 0.2-μm YG "Fluoresbrite" carboxylate beads (Polysciences, Inc.). After 48 h they were removed, rinsed in seawater, fixed for 24 h in 10% formalin, decalcified for 7 days in cold 5% disodium EDTA, embedded in gelatin, and cut as 20-μm frozen sections. The distribution of the beads was then observed by epifluorescent UV microscopy.

Further tests were conducted to see if madreporite oblation would lead to diminished functions. Specimens (groups of 6) were anesthetized by a brief immersion in 8% MgCl in tap water. The region of the madreporite and stone canal (identified by the pigmentation pattern) was then surgically destroyed and sealed with a small

Figure 7. Section just above Figure 6, showing the primary madreporite pore (PP) in the crevice of the genital bursal slit. A, ampulla.

Figure 8. Section located 40 μm higher than Figure 7. The ampulla (A) is opening into the bottom of the right axial sinus (RAS), opposite to the opening of the stone canal (SC), which contains long flagella. The lower axial organ (AO) can now be seen in the left axial sinus (LAS).

Figure 9. Section located 60 μm above Figure 8, showing typical form of the axial complex in this region. nThe axial organ (AO) here is thin and contains only a few cell nuclei. It is applied closely to the flagellated columnar cells of the stone canal (SC). LAS, left axial sinus; RAS, right axial sinus.

Figure 10. Section about 100 μm higher than Figure 9. At this level the axial complex lies between the perivisceral coelom (PC) and the interradial muscle (IM). The axial organ (AO) gives off a pair of genital hemal strands (GHS), surrounded by extensions of the left axial sinus (LAS). The right axial sinus (RAS) is beginning to expand.

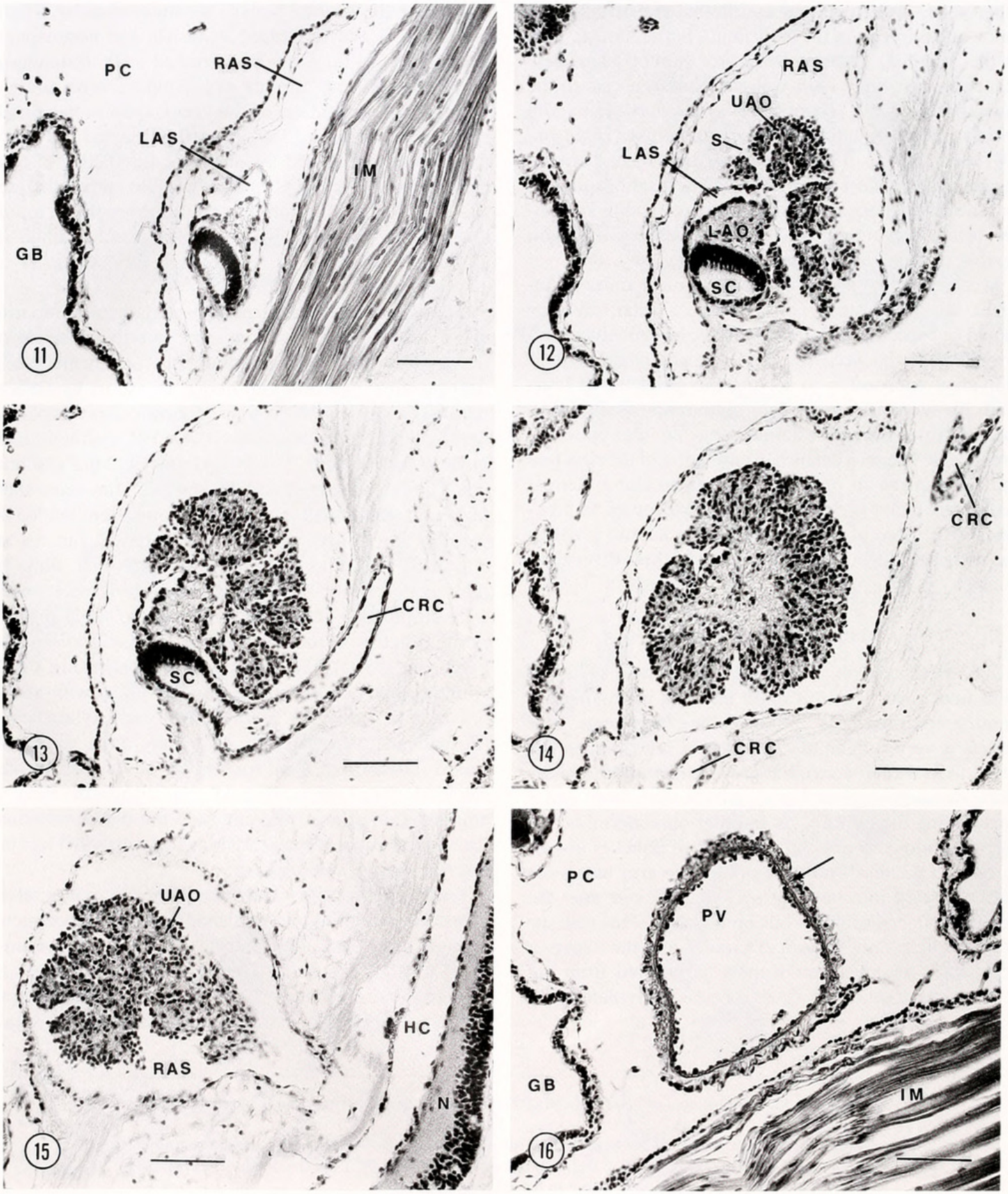


Figure 11. Section about 170 μm higher than Figure 10. In this region the right axial sinus (RAS) has expanded laterally and nearly surrounds the rest of the axial complex. It is separated by only a very thin double peritoneal membrane from the extension of the perivisceral coelom (PC) that accompanies the large interradial muscle (IM). GB, genital bursa; LAS, left axial sinus.

cement plug. Control animals were treated similarly, but a comparable wound was made through an oral shield on the opposite side of the body from the madreporite. All of the animals recovered from the immediate effects of the anesthetic in about an hour. They were then observed for 7 days to see if their tube feet would extend and make normal stepping motions, and if the specimens would right themselves when overturned. The animals also were periodically blotted and weighed to see if weight variations could demonstrate any changes in body fluid content. The cement plugs were usually lost after about the second day as growing tissue sealed the wound. Otherwise, all animals except one, which fractured in two, appeared healthy throughout the study period.

In a final set of observations, groups of three specimens (tests and controls) previously operated on in the above manner were placed in seawater to which sufficient dextran (M.W. 5300) had been added to raise the osmotic concentration 20 mosmoles/kg. The purpose of this approach was to try to counter any osmotic inflow by using an osmolyte that should be neither permeable nor chemically harmful to the integument. The animals were observed and weighed as before until, on the third day, the experiment was terminated.

Results

As previously noted madreporitic pores cannot be seen in intact specimens of *Ophioderma appressum*. Examination of the serial sections revealed that they are small and hidden by the outer edge of one of the oral shields. It was found, however, that their position on the body could usually be determined by the slightly greater amount of pigmentation on that oral shield compared to the others, or if the pigmentation were absent, by the dark shadow of the ampullary complex showing through the translucent oral shield (Figs. 1, 2). From the oral perspective, the pores lie just within the crevice of the central genital bursal slit on the distal left side of the oral shield. Normally, water currents could be seen passing into the peripheral slits of the genital bursae and exiting through the central ones. Thus, while the animals lie on the silty bay bottom, the madreporitic pores are mainly bathed by seawater that has passed through the genital bursae from the presumably cleaner source at the edge of the disk. The water currents within the genital bursae are maintained by cilia as well as by rhythmical expansions and contractions of the whole upper part of the disk.

Structure

The organization of the entire water vascular system is shown in Figure 3, and a more detailed representation of the complex madreporite-axial structure in Figure 4. Figures 5–18 show views of some of the serial sections from which these two diagrams were developed (differences between the two specimens studied were negligible). To avoid confusion in the following descriptions, the photomicrographs have been reversed in printing to compensate for the normal optical inversion of the compound microscope.

The primary opening into the system from the exterior is a 10–15 μm diameter pore located in the folded edge of the central genital bursal slit, just under the lip of the oral shield (Fig. 7). Several undulations occur in the layer of cuboidal cells that line the pore passage; raising the possibility that more pores might develop in specimens more fully grown then the two studied. A second pore of nearly the same diameter is located about 150 μm lateral and slightly lower than the first (Fig. 6). Its duct points towards the first and away from the stone canal. This orientation suggests that the secondary pore may be a rejection pathway, but there is no way of verifying that.

Both pores lead to a discrete lobe of the madreporite "ampulla." This spacious chamber lies just under the surface of the oral shield. It is lined with a distinctive cuboidal epithelium, and it has several regions separated by sharp angles (Figs. 5–8). After making tortuous turns, it opens broadly into the lower end of the "right" axial sinus (Fig.

Figure 12. Section 70 μm above Figure 11. The upper axial organ (UAO) has formed large cellular lobes that hang down into the right axial sinus (RAS). The left axial sinus (LAS) penetrates the axial organ with slit-like spaces (S) that continue through to the surrounding right axial sinus. The lower axial organ (LAO), containing only scattered cell nuclei, still lies close to the stone canal (SC).

Figure 13. Next section above Figure 12. The stone canal (SC) here connects to the circumoral ring canal (CRC). The cellular lobes of the axial organ and the slits between them connecting the two parts of the axial sinus are quite evident.

Figure 14. Section 40 μm above Figure 13, showing the top of the axial organ forming its cellular lobes. Parts of the undulating circumoral ring canal (CRC) are visible on either side of it.

Figure 15. Section 60 μm above Figure 14, showing extensions from the upper axial organ (UAO) and the right axial sinus (RAS) toward the circumoral hyponeural coelom (HC) and the oral hemal ring (not visible). N, nerve ring.

Figure 16. A polian vesicle (PV) lying in the perivisceral coelom (PC) next to an interradial muscle (IM). Note the heavy wall of the vesicle and its elastic membrane (arrow). GB, genital bursa.

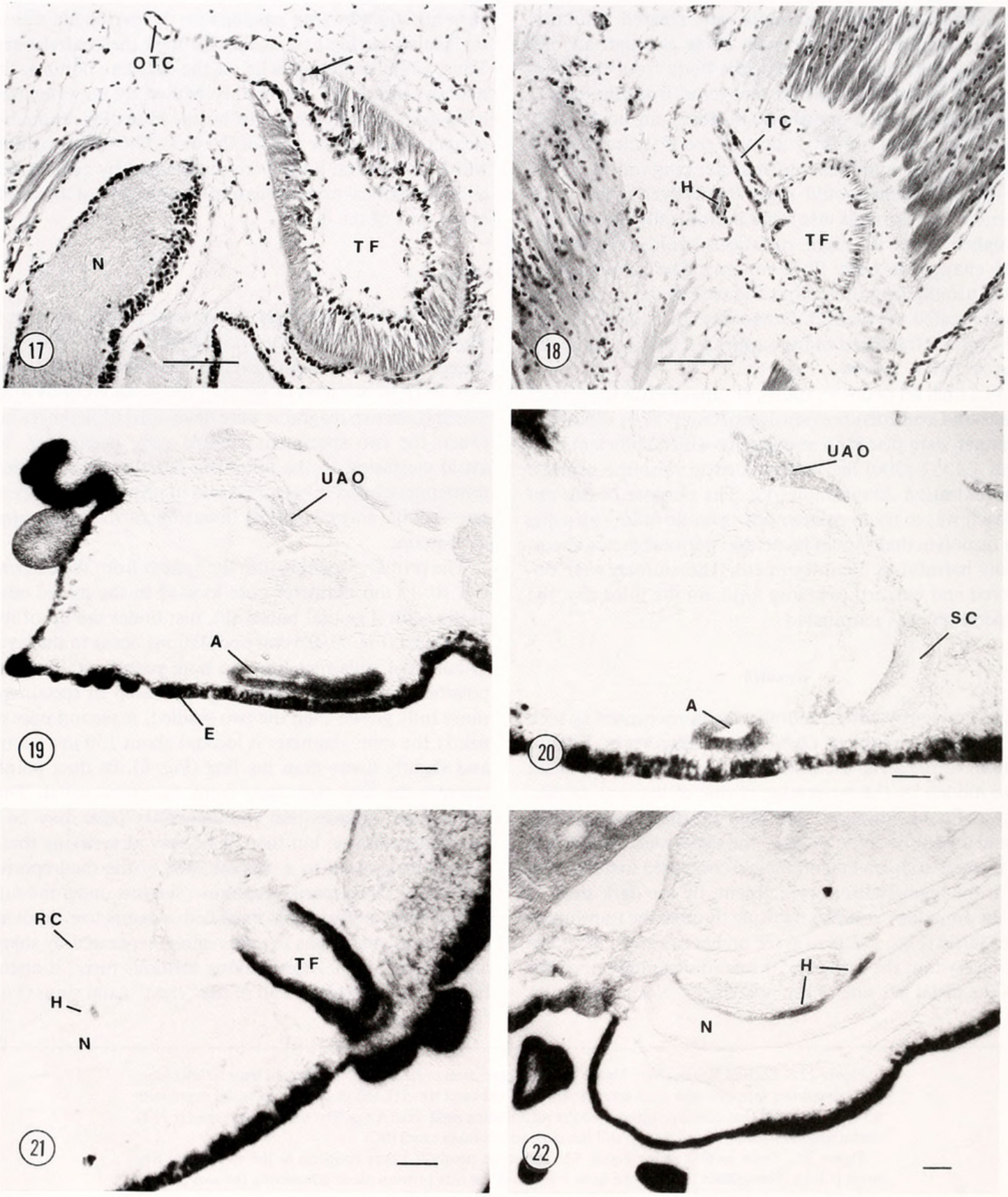


Figure 17. The connection of an oral tube foot (TF) with its canal (OTC) from the circumoral ring canal. There is a muscular restriction at the neck (arrow), but no valve. N, nerve ring.

Figure 18. A transverse canal (TC) in an arm connecting to a tube foot (TF). It has a well-developed valve. A strand of hemal tissue (H) connecting the tube foot to the radial hemal vessel lies nearby.

8). Opposite this opening is the entrance to the lower end of the stone canal (Fig. 8), which thus does not directly connect with the ampulla, but rather with the right axial sinus. On the far side of the stone canal (and attached separately to it) is the "left" axial sinus, a somewhat smaller chamber. It has no opening at the lower end, but contains the axial organ (part of the hemal system), broadly attached to the wall of the stone canal. Both parts of the axial sinus are lined with a thin, simple squamous peritoneum. The cells forming the stone canal stain densely. They are cuboidal adjacent to the right axial sinus, and columnar next to the axial organ. Long flagella extend from the columnar cells and reach nearly across the 15–20 μm width of the somewhat flattened lumen (Figs. 9–12). The close proximity of the hemal tissue to the position of the flagellated cells suggests that hemal fluid might supply the high levels of energy nutrients that must be needed by these cells to produce a current within the stone canal.

The four structures (the two separate parts of the axial sinus, the stone canal, and the axial organ) all rise in a long arch towards the ring complex located high up in the mouth frame. As they rise, they sweep over the large interradial muscles (Figs. 10, 11). About one third of the way up, the axial organ gives off the genital hemal strands surrounded by extensions of the left axial sinus (Fig. 10). These are thought to supply nutritive materials to the gonads (Walker, 1982; Byrne, 1988, 1989). Above that point, the right axial sinus begins to expand and stretch out laterally (Fig. 11). It comes to surround, almost completely, the other three structures, and medially it forms a long thin boundary with the perivisceral coelomic extension that partially encases the interradial muscle. On the distal side, it is separated from the perivisceral coelom by a thin layer of connective tissue (Figs. 11–15).

After the right axial sinus begins its expansion, the left axial sinus splits into a series of slit-like passages that enter the axial organ, which now becomes highly cellular and bulges out like a cauliflower (Figs. 12, 13). These passages then open extensively into the right axial sinus, completing the connection between the two portions of that cavity. At its upper end, the axial organ becomes more solid again (Fig. 14) and gives off an extension to the oral hemal ring (Fig. 15). This extension is surrounded by a portion of the right axial sinus, which appears to connect with the hyponeural (perihemal) compartment that lies adjacent to the nerve ring. This connection is not a spacious opening, but a complex grouping of peritoneal cells and hemal tissues that were difficult to resolve in the preparations studied.

At the same level in which the two parts of the axial sinus join, the top of the stone canal bends over and joins the ring canal that encircles the mouth (Fig. 13). The ring canal, like the other canals of the water vascular system, is lined with squamous cells surrounded by an elastic membrane, fibrous connective tissue, and a few muscle cells (Figs. 13, 14). In the other four interradii, the ring canal gives off canals that open into bulbous polian vesicles. These lie in the perivisceral coelom next to the interradial muscles. Their walls possess a conspicuous elastic membrane like that of the water canals (Fig. 16). In each radius, the ring canal gives off three branches—a radial canal that descends and runs out the arm, and, well to either side of it, canals that connect to upper and lower oral tube feet that lie horizontally between the jaws, within the mouth frame. There are no valves between the oral tube feet and their connecting passages, although sphincter muscles may be able to restrict the openings (Fig. 17).

Along the arms, transverse canals extend in pairs from the radial canal. At the opening of each canal into a tube foot, there is a well-developed valve that holds fluid within the appendage (Fig. 18). Accessory elastic vesicles, such as Woodley (1967) described as extending from the radial canals of *Amphiura filiformis*, were not seen. When the tube foot retracts, it pulls up into a surrounding sheath that is then closed over by two or three flattened spines. When it extends, it slides out of this sheath as a unit and then stretches out as a dexterous tentacle. Reiger and Lombardi (1987) have reported on the ultrastructure of the wall of the tube feet of *Ophioderma brevispinum* and other species.

The radial canals lie in loose connective tissue in the lower portion of the arm. Below them (above the nerve cord) are extensions of the hemal tissues and hyponeural

Figure 19. An autoradiograph of an unstained radial section of the disk of an animal exposed to ^{14}C-amino acids in seawater for 8 h. Note the considerable darkening (radioactivity) in the ampulla (A). A small amount of label, perhaps from ingestion, is in the upper axial organ (UAO). The exposed epidermis (E) is intensely labeled.

Figure 20. Autoradiograph of a section near that of Figure 18. Label is seen in the ampulla (A), lightly in the stone canal (SC), and a bit more intensely in the upper axial organ (UAO).

Figure 21. Autoradiograph of a transverse section of an arm of the same specimen as Figures 19, 20. No radioactivity is seen in the radial canal (RC), but it is found clearly in the radial hemal vessel (H). Some label may be in the lining of the tube foot (TF), but there is strong background from the heavily labeled epidermis. N, radial nerve cord.

Figure 22. Autoradiograph of a radial section of arm base of the same specimen as others. Note the high level of uptake in the radial hemal vessel (H). N, radial nerve cord.

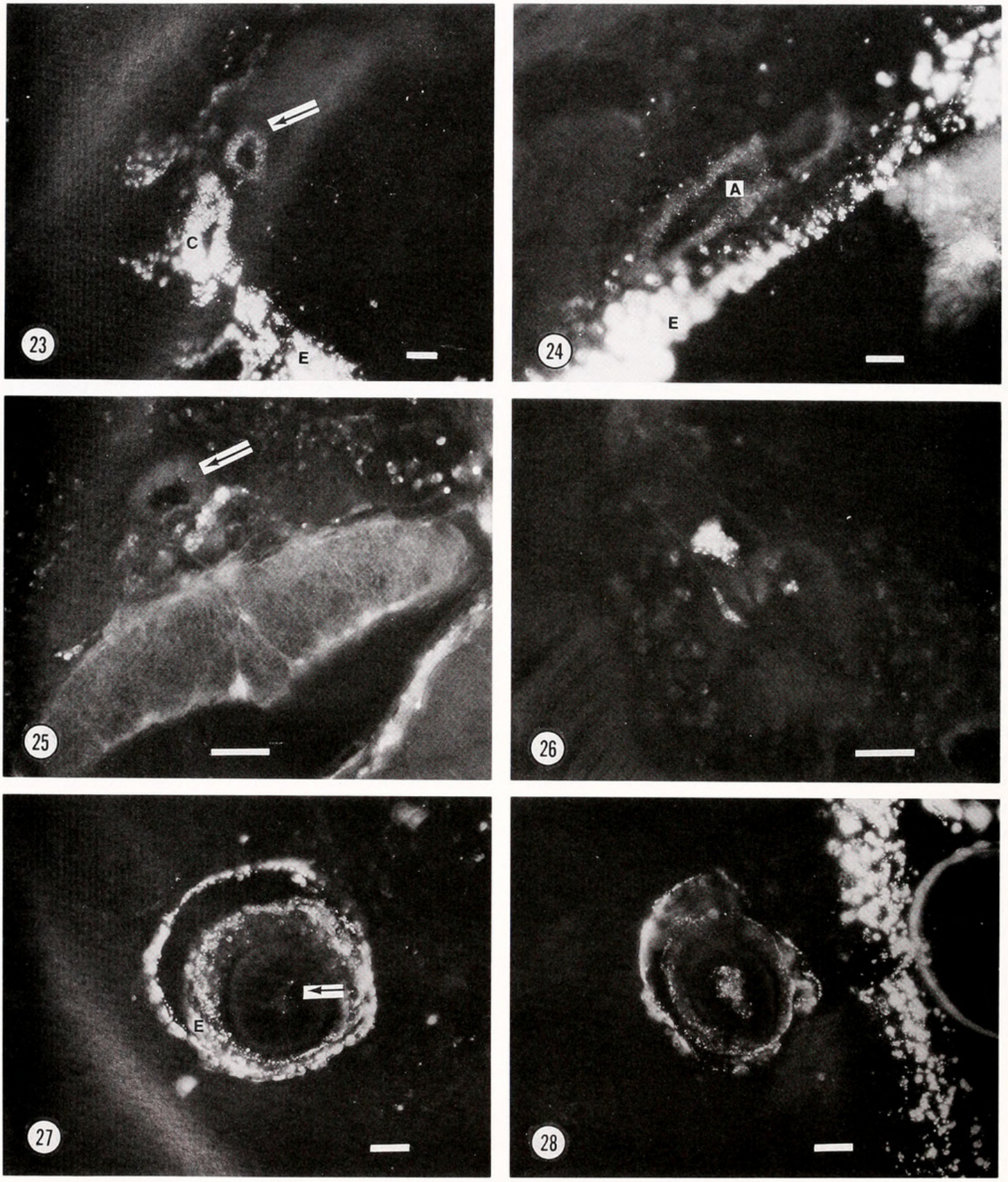


Figure 23. This and the next five figures show epifluorescent views of specimens exposed for 48 h to fluorescent microbeads in seawater. Here a pore canal (C) extends towards the ampulla (arrow). Numerous beads are in the pore canal and epidermis (E); smaller numbers are in the cellular lining of the ampulla.

Figure 24. A view of the ampulla (A) just above the epidermis (E) of the oral shield. A few beads are found in the ampullary chamber and some in its cellular lining.

Table I

Weight variations (g) of Ophioderma appressum *following destruction of the madreporite area, and control animals with a comparable injury to another site*

Specimen	Start	1 day	2 days	3 days	4 days	7 days
Madreporite destroyed						
1	1.14	1.12	1.11	1.07	1.07	1.09
2	1.01	1.01	1.02	1.02	1.07	1.04
3	1.29	1.28	1.29	1.26	1.32	1.28
4	1.49	1.50	1.57	1.50	1.57	1.55
5	0.61	0.61	0.60	0.62	0.66	0.63
6	1.10	1.09	(fragmented)			
Controls						
1	0.98	0.98	0.97	0.97	0.98	0.94
2	1.97	1.96	1.98	1.97	1.99	1.96
3	0.83	0.83	0.87	0.85	0.83	0.83
4	0.58	0.50	0.48	0.50	0.50	0.50
5	0.59	0.56	0.57	0.58	0.58	0.59
6	0.64	0.59	0.58	0.59	0.58	0.59

coelomic spaces. Hemal tissue extends laterally to each tube foot (Fig. 18) where it appears to join, near the valve, with the middle connective tissue layer of the appendage.

Tracer studies

Autoradiographs of animals placed for 8 h in ^{14}C-amino acid mixture showed intense incorporation of the tracer within all exposed epidermal cells including those of the tube feet and their sheaths. This result was expected from earlier studies (Ferguson, 1967; Fontaine and Chia, 1968). The concern here was whether the tracer could demonstrate seawater entry into the water vascular system. That was the case, as notable radioactivity was found in the lining cells of the madreporite ampulla (Figs. 19, 20). This labeling diminished rapidly up the stone canal (Fig. 20). [In animals fed labeled surf clams, radioactivity was found abundantly in the hemal tissues, but not extensively in the water vascular passages (Ferguson, 1985)]. No label was visible within the radial canals, and amounts in the lumen of tube feet were low and not readily distinguished from background (Fig. 21). The radial hemal vessels accumulated considerable amounts of label (Figs. 21, 22), and a small amount was seen in the upper, cellular portion of the axial organ (Fig. 19). These accumulations could have come from an oral route (*cf.* Ferguson, 1985).

Fluorescent microbeads were employed as a means of more directly following the flow of seawater into the water vascular passages. Unlike the labeled amino acids, these beads should not easily penetrate membranes or become rapidly depleted by cells as they take up nutrients from the fluid flowing by them. However, the entry of beads into the body must overcome the animal's natural defenses against foreign particles. A very large number of beads was used in the medium with the expectation that a proportion of them would get through if bulk fluids were indeed entering the body through the pores. After 48 h of exposure, fluorescent beads were found in many water vascular passages, but not in great abundance (Figs. 23–28). Beads were seen moderately distributed within the lining of the ampulla (Figs. 23, 24). Some were found free in the radial canals (Fig. 25) and in the tube feet (Fig. 27). More commonly, beads were found engorged in clumps of coelomocytes. These often accumulated in the lateral canals just before the valves (Fig. 26), or within the lumen of tube feet (Fig. 28). Many tube feet, however, could be seen without any beads within them. Coelomocytes bearing many beads collected in the hemal tissues of the arms, especially between the radial hemal vessels and the tube feet, and beneath the radial canals (Fig. 25). A few clumps of coelomocytes containing beads were found in the perivisceral coelomic spaces of the arms. All exposed epidermal areas were filled with beads, including the areas just outside the madreporitic pores (Fig. 23) and the sheaths of the tube feet (Figs. 27, 28).

Experiments

Because the tracer studies showed that seawater must enter the madreporitic pores, at least in small quantities, a study was made to see whether that uptake was essential to the animals. It was found that surgical obliteration of the madreporite, ampulla, and lower stone

Figure 25. Transverse section through the ambulacrum of an arm. Several discrete microbeads lie in the radial canal (arrow). Below the arrow is part of a hemal strand extending towards a tube foot. It contains many coelomocytes engorged with the beads. Most of the other fluorescence is natural.

Figure 26. A clump of coelomocytes engorged with beads lying in a transverse canal just before the valve. Two or three coelomocytes containing beads are nearby, within the tube foot.

Figure 27. Transverse section through a tube foot lying in its sheath. A number of the fluorescent beads are seen in the lumen (arrow). The epidermal tissue (E) of the tube foot and its sheath contains many beads that were taken up directly from the seawater.

Figure 28. Transverse section through another tube foot. This one contains, in its lumen, many free beads as well as coelomocytes engorged with beads.

Table II

Weight variations (g) of Ophioderma appressum *in seawater raised 20 mosmoles/kg with dextran (5300 M.W.); specimens with madreporites destroyed and control animals with a comparable injury to another site*

Specimen	Start	4 hours	1 day	2 days	3 days
Madreporite destroyed					
1	1.09	1.04	1.05	1.06	1.12*
2	1.28	1.24	1.23	1.23	1.28*
3	0.65	0.61	0.60	0.60	0.69*
Controls					
1	0.69	0.66	0.64	0.64	0.65*
2	0.45	0.43	0.41	0.40	0.42*
3	1.09	1.05	1.01	1.02	1.04*

* Animals abnormal—arched up and rigid.

canal had very little effect on tube foot function for at least a week. For the first two or three days, tube foot activity declined somewhat, especially in the oral tube feet, but there was no consistent difference in observed behavior when compared to controls. All tube feet could extend and bend, and when animals were overturned, righting movements involving the arms were unaffected. The number of tube feet active at any one moment may have diminished, but with the diversity of individual behavioral responses demonstrated by the specimens, that could not be quantified. Nor were there any consistent variations in body weights that would reflect fluid volume changes (Table I). Both test animals and controls varied a few percent from day to day, but not significantly.

If tube foot inflation and body fluid content are maintained by osmotic elevation, as by a potassium ion pump in the tube feet (*cf.* Prusch, 1977), the mechanism could be sensitive to elevated colloidal osmotic pressure in the medium. To test this possibility, animals with obliterated madreporites, and controls, were placed in dishes of seawater in which the osmotic levels had been elevated a modest amount (20 mosmoles/kg) with dextran (5300 M.W.). In both groups the immediate effect was a small loss in weight (2 to 4%) over the first few hours and then stability within a normal range (Table II). For the next two days there was no diminishment of tube foot function or other observable effects. On the third day, animals in both groups showed some arching rigidity of their arms, rather similar to that seen previously in animals placed in ionically altered seawaters. At that point the experiment was terminated.

Discussion

This study has shown that *Ophioderma* has a complex water vascular system, but one in which the passages from the exterior (the madreporite pores) appear to be of minor importance compared to provisions for internal recirculation of fluid *via* the axial sinus and its extensions. Although it was shown that seawater routinely enters the pores, it must do so only in small quantities. Under the laboratory conditions employed, this uptake seemed to provide little advantage. Perhaps under the stresses of the natural environment uptake may be important in some circumstances, but such conditions have yet to be discovered. Fluid might also exit the pores when pressures in the system become too great.

In contrast to the minimal madreporite of *Ophioderma,* the madreporites of asteroids have many pores and complex arrangements of ciliated gutters to keep them free of suspended foreign particles (Ferguson and Walker, 1991). In one study on *Echinaster graminicola,* seawater uptake through the madreporite was equal to about 5.5% of the animal's body weight per day; and more than half of it went into replacing the general body fluid (Ferguson, 1989). The two small pores of *Ophioderma,* located very inconspicuously at the edge of a genital bursal slit, clearly cannot allow much seawater into the system. Reduced inflow would alleviate contamination from the silty environment in which the animals normally live. Further, the genital bursa might protect the pores from silt, as the asteroid gutters do. Likewise, the complex form of the ampulla probably plays a sanitary role, because many beads were seen taken up by its cells.

The stone canal itself is fairly well developed. It is smaller in diameter than those of asteroids, and it does not have the internal ridges that make a larger diameter tube more efficient as a ciliary pump. It also does not have as much bony ossicle material, needed by a larger structure for strength. However, for the much smaller body size of *Ophioderma,* the stone canal seems proportionately scaled. Its lumen is somewhat flattened, which allows the flagellated cells to be very efficient in sweeping fluid through the canal.

As noted, the stone canal does not connect directly with the ampulla, but rather with the right axial sinus. If fluid flows down this sinus into the stone canal, as it appears to, it would be drawn from three sources: (1) the left axial sinus (and the genital perihemal vessels), through the slits of the axial organ; (2) the circumoral hyponeural (perihemal) coelom (and its connections with the radial hyponeural coelomic spaces of the arms), over the surface of the axial organ; and (3) through the delicate peritoneal membranes separating much of the axial sinus from the perivisceral coelom. I conclude, then, that most of the fluid pumped by the stone canal is coelomic—not seawater flowing in through the two madreporitic pores. As the fluid flows over the axial organ it is probably purified, and it probably gives up

nutritive materials to form hemal fluid. [The transport of nutritive material by hemal systems was previously described (Ferguson, 1984, 1985)].

The fluid pumped by the stone canal then passes through the water vascular canals, and most of it eventually travels out the arms. Some is diverted to the oral tube feet and the polian vesicles, which among other functions, probably collectively serve as a general reservoir and pressure stabilizer for the system. In the arms, the fluid passes through the valves into the tube feet only when the hydrostatic pressure of the system surpasses that maintained in the appendages. The tube feet also might be kept inflated by osmotic inflow. Based on the observations on other animals by Robertson (1949), Binyon (1976b), Prusch (1977), and Ferguson (1990b), the tube feet likely contain a higher osmotic pressure than the surrounding seawater. In the present experiments, however, the tube feet failed to collapse when the external osmotic pressure was increased with dextran, though by the third day the treatment appeared to produce pervasive detrimental effects on the bodies of the animals. Although the water vascular vessels can deliver fluid to the tube feet, they may be equally valuable in providing a more general circulatory flow by permeation to all the tissues of the lower arms, and return *via* the hyponeural spaces. Additional circulation is achieved by the perivisceral coelomic passages.

When compared with the body cavities of asteroids, the perivisceral coelom of *Ophioderma* is not large. Within the arms it consists mostly of canals that expand into larger spaces between the vertebral-like ossicles. Asteroid perivisceral fluid is kept under low, but positive, hydrostatic pressure (Ferguson, 1988). That cannot be the case in *Ophioderma*. Distinct "breathing" motions of the aboral disk alternately stretch and compress the coelomic space, and that movement pumps seawater in and out of the genital bursae. The ventilation must produce negative coelomic pressures that should lead to the accumulation of fluid in the coelomic space by filtration. Pressure from pumping by cilia in the genital bursae would have the same effect. [Net negative coelomic pressures have recently been described in sea urchins by Ellers and Telford (1992), but these are produced by a different mechanism.] It appears, then, that *Ophioderma* has little need to take up seawater through its madreporite either to support its tube feet or to maintain its perivisceral coelomic fluid, and it has a limited ability to do so. Asteroids, on the other hand, often do tend to lose large amounts of fluid from their bodies and must replace it. For them, the madreporite system (together with the Tiedemann's bodies) is a much more important and well-developed mechanism.

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