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Cellular Morphology of *Callyspongia diffusa*

Abstract

Callyspongia diffusa, an Indo-Pacific sponge, shows a cell-mediated cytotoxic rejection response to artificially grafted allogeneic tissues. Basic to the understanding of this cellular interaction is a characterization of the cells normally found within this species. Histological analysis of the tissues of this sponge reveals pinacocytes, choanocytes, archeocytes, spherulous cells, acid mucopolysaccharide-positive cells, acidophilic granular cells, globoferous cells, sclerocytes, and germ cells.

Many different cell types from various sponge species have been morphologically characterized. Some distinct cell types, such as pinacocytes, choanocytes and archeocytes are found in all sponges. However, other cell types, including many of the mesohyl cells, are not found in all sponge species. Consequently, conclusions drawn from cytological analyses of one sponge cannot be assumed to apply to all other species.

Recently, *Callyspongia diffusa*, an Indo-Pacific sponge, has been used to investigate allograft rejection responses (Hildemann et al., 1979, 1980; Bigger et al., 1981, 1982; Johnston et al., 1981; Johnston and Hildeman, 1983; Smith and Hildemann, 1986 a,b). Because this response is a cell mediated cytotoxic reaction to non-self tissues, an understanding of the normal component of cell types within this species becomes important. In the present study, the morphological identifications of the cell types normally found within *C. diffusa* are presented. They include pinacocytes, choanocytes, archeocytes, spherulous cells, acid mucopolysaccharide positive (AMP+) cells, acidophilic granular cells, globoferous cells, sclerocytes, and germ cells.

Materials and Methods

Callyspongia diffusa is a brilliant purple sponge found on reef crests in Kaneohe Bay, Oahu, Hawaii. Several large

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specimens were collected from the fringing reefs around Coconut Island and from a patch reef near the Kaneohe Yacht Club. Sponges were maintained in unfiltered running seawater at the Hawaii Institute of Marine Biology (University of Hawaii) and used within 4 days of collection. Sponges were fixed overnight in Bouins fixative, followed by an extensive rinsing in filtered seawater and then in tap water. The specimens were routinely dehydrated and embedded in paraplast (Monoject Scientific, Dehand, FL). The spicular skeleton of the sponge made sectioning at room temperature impossible. Consequently, to harden the paraplast, the rough trimmed blocks were frozen (–25°C) and cut at 6–8 μm in a cryostat. Test sections were stained with 1% toluidine blue before deparaffinizing to assess tissue orientation. Selected sections were routinely stained with hematoxylin and eosin (H & E), periodic acid Schiff (PAS), alcian blue with fast red as a counter stain, and Masson's trichrome stain. The sections were photographed on Plus-X film in a Zeiss or Olympus photomicroscope.

Results

TISSUE ORGANIZATION. The tissues of *Callyspongia diffusa* are supported by the anastomosing, spongin-encased, spicular skeleton. The body of the animal is divided into two general regions: the choanosome and the ectosome (Figure 1). The choanosome encompasses the inner regions and contains the choanocyte chambers or water pumping structures, the smaller aquiferous canals, and the mesohyl. The ectosome is located in the superficial regions and includes the exopinacoderm or outer layer of cells, and the subdermal spaces located just below the exopinacoderm. The ectosome normally contains few choanocyte chambers, but in *C. diffusa* there are many areas in which the choanosome extends between the subdermal spaces to reach the outer covering.

The aquiferous canal system is an interconnection of incurrent and excurrent canals, at the junction of which are found the choanocyte chambers (Figure 1). Normal physiological functions of this system include food pro-

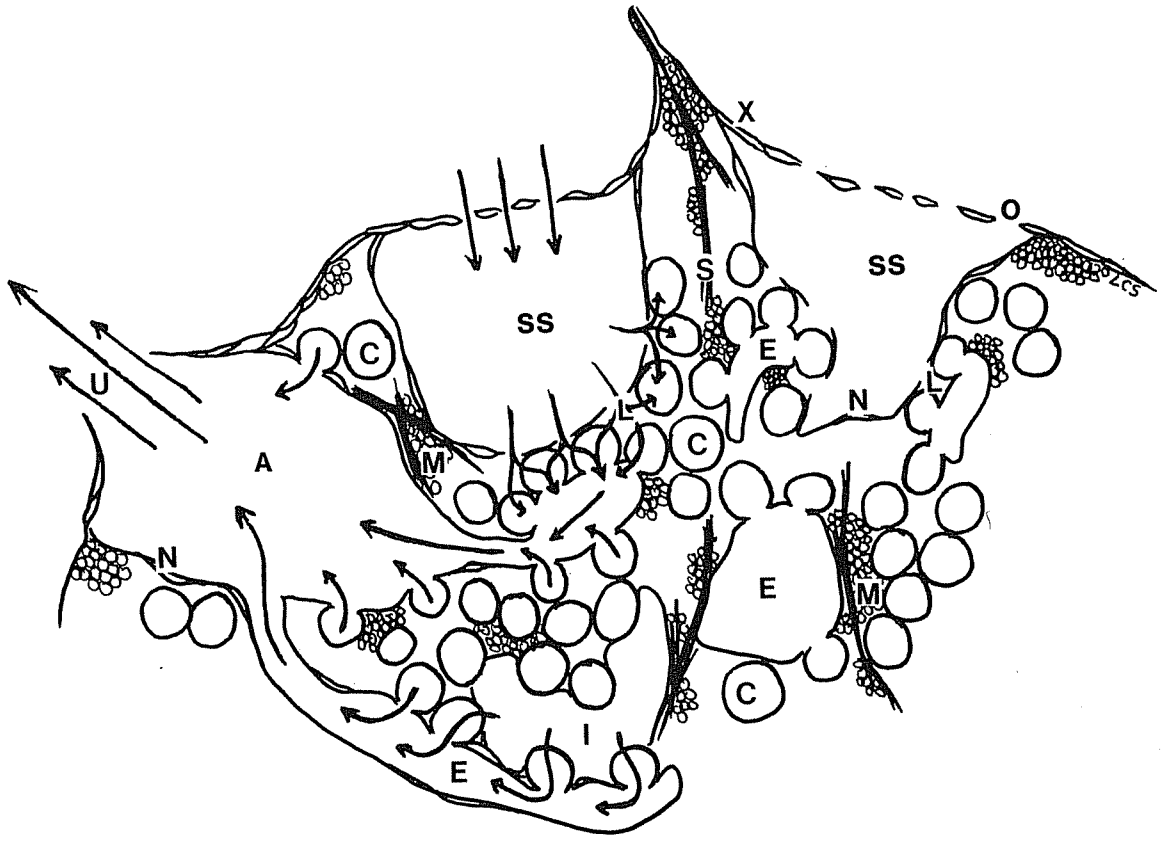


Figure 1. Pathway of water flow through *Callyspongia diffusa* begins as water *(arrows)* enters through incurrent pores or ostia, often grouped together in sieve-like complexes in exopinacoderm. From ostia, which lead into subdermal spaces and then to incurrent canals, water passes into lacunar spaces and then between cell bodies of spherically arranged choanocytes. After passing through chamber, water enters excurrent canal. Several excurrent canals join to form atrium located below excurrent pore or oscula. *A*, atrium; *C*, choanocyte; *E*, excurrent canal; *I*, incurrent canal; *L*, lacunar space; *N*, endopinacocyte; *O*, ostia; *S*, skeleton; *SS*, subdermal space; *U*, osculum; *X*, exopinacoderm.

curement, elimination, gas exchange, and gamete release (Simpson, 1984). An excellent account of the pathway of water flow in *Callyspongia diffusa* has been presented by Johnston and Hildemann (1982), and is briefly reviewed in Figure 1.

Sponges lack defined organ systems. The only anatomically discrete tissue is the epithelium, which is composed of two cell types: pinacocytes and choanocytes. These cells cover the sponge surface, line the aquiferous canal system, and are in constant, direct contact with the ambient water. The remaining cells, which are located in the mesohyl, are bounded on all sides by the epithelium and constitute the true internal regions of the sponge body. The cell types characterized in *C. diffusa* are presented in Tables 1–3.

PINACOCYTES. The pinacocytes cover the external and internal surfaces of the sponge. They are flattened, fusiform, slightly basophilic cells with small, centrally located nucleolate nuclei (Figure 2), and according to Johnston and Hildemann (1982) are irregular in shape and about 20 μm in diameter. The exopinacocytes line the outer covering of the sponge while the morphologically identical endopinacocytes line the aquiferous canal system. The external surface layer, the exopinacoderm, is composed of a double layer of cells: the outer exopinacocytes and the inner endopinacocytes that line the subdermal spaces and atria. These cells may line up edge to edge, or they may overlap. The exopinacoderm located over the subdermal spaces has many pores, or ostia, grouped together in sievelike structures that allow water to enter the aquiferous system. Each ostium appears to be an intracellular structure of a single porocyte (Johnston and Hildemann, 1982).

CHOANOCYTES. The spherical choanocyte chambers are located at the points where the incurrent and excurrent canals intersect. Choanocytes are rounded and slightly basophilic cells with condensed, basally located nuclei (Figure 3). Each choanocyte has a single flagellum oriented toward the center of the chamber which beats to create a flow of water through the aquiferous system. Around each flagellum, there is a ring or collar of microvilli that acts as the fine filter for collecting food particles brought in by the water current. (The collar and flagella on the choanocytes are not easily visualized by methods employed here; see Johnston and Hildemann, 1982). It is surprising that these cells, which function to capture food by filtration and phagocytosis and normally exhibit many phagosomes (Willenz, 1980), contain no cytoplasmic inclusions. Lack of inclusions may be a result of recent improvements in water clarity in Kaneohe Bay, which have reduced particulate food supplies.

MESOHYL. The mesohyl is a loose association of cells bounded on all sides by pinacocytes or choanocytes. The several cell types found in this region include archeocytes, several types of spherulous cells, acid mucopolysaccharide positive (AMP+) cells, acidophilic granular cells, globoferous cells, sclerocytes, and germ cells.

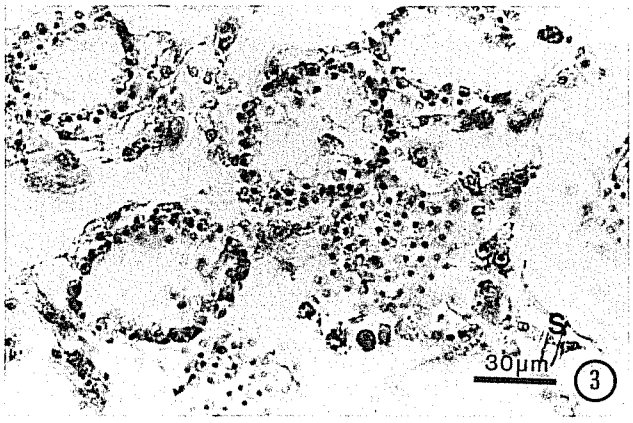
ARCHEOCYTES. Archeocytes are easily identified by their large nucleolate nuclei (Figure 4). These cells are very heterogeneous; the cytoplasmic basophilia ranges from almost clear to dark blue and the cytoplasmic inclusions, which are also basophilic, can be numerous or completely absent. The archeocytes, which comprise 84.4% of the total cells in the mesohyl, are generally considered to be amoeboid, phagocytic cells.

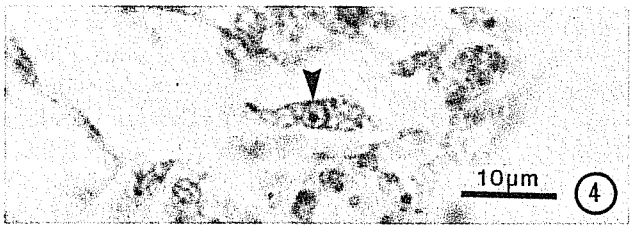
SPHERULOUS CELLS. The spherulous cells have small, condensed, anucleolate nuclei that are located in the center of the cell. The cytoplasm is filled with large spherical inclusions (the basis for the cell name). There are three types of spherulous cells in *Callyspongia diffusa,* which are based on differences in spherule-staining properties (Table 1). By H & E, they are acidophilic, neutrophilic, and mixed. The acidophilic spherulous cells (Figure 5), which consist of 7.4% of the total mesohyl, are found scattered throughout the sponge and in clusters (containing 8.9 ± 5.6 cells) located just below the exopinacoderm where the mesohyl reaches the surface (Figure 6). This distribution has been noted in other species (Bretting and Königsmann, 1980; Bretting et al., 1983). The neutrophilic spherulous cells, which compose 7.8% of the mesohyl, are also scattered throughout the sponge, but they are never found clustered in groups, nor do they appear to release their spherules (Figure 7). The rare, mixed spherulous cells are usually found on the endopinacoderm (Figure 8).

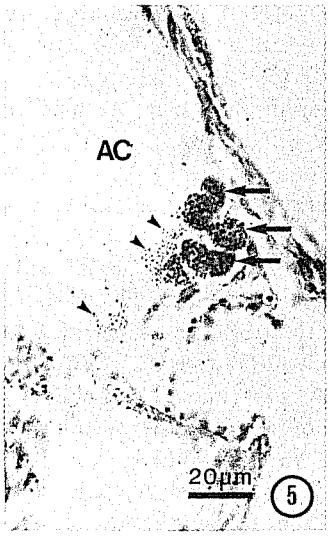
OTHER MESOHYL CELLS. Several other rare mesohyl cell types include AMP+ cells, acidophilic granular cells, sclerocytes, and globoferous cells. The large AMP+ cells are best characterized by intense cytoplasmic staining with alcian blue, indicating the presence of acid mucopolysaccharides in the cytoplasm (Figure 9). The nuclei are small and in some cells appear stellate. These cells are located randomly within the mesohyl and are also found slightly flattened against the endopinacoderm inside aquiferous canals. Acidophilic granular cells have irregular shapes and small but prominent nucleolate nuclei, and are full of small acidophilic granules (Figure 10). They are usually located on the endopinacoderm, although they have been noted in the mesohyl. Globoferous cells, found rarely in the mesohyl, are identified by a large clear eccentric area and large basophilic spherical vacuoles that obscure the nuclei (Figure 11). Spicule secreting sclerocytes (Simpson and Vacaro, 1974), have only been identified in *Callyspongia diffusa* in preparations of dissociated cells by

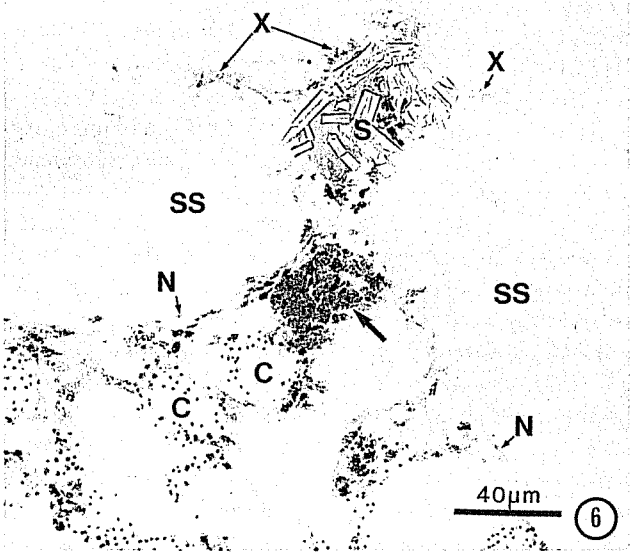
the presence of intracellular microscopic spicules (not illustrated). This cell type has not been noted in histological preparations, perhaps because the growing tips of the sponge branches were not used in the experimental procedures.

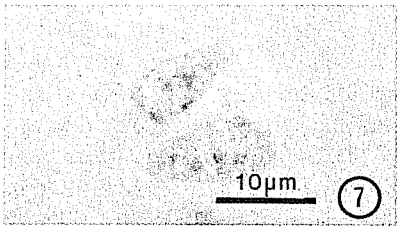
GERM CELLS. In addition to the somatic cells, *Callyspongia diffusa* contains sperm, eggs, and embryos (Table 2). In the male sponge, the sperm are found in cysts located throughout the mesohyl, but always near an aquiferous canal (Figure 12). Within each cyst, cell division appears

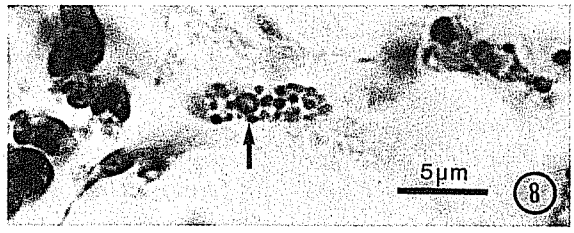












Figures 2–8. Histology of *Callyspongia diffusa: 2,* pinacocytes in an "en face" view (arrowheads show mixed spherulous cells), H & E stain; *3,* choanocyte chambers located within choanosome; flagellae and microvillar collars not visible; H & E stain; *4,* an archeocyte shows prominent nucleolate nucleus *(arrowhead),* H & E stain; *5,* acidophilic spherulous cells *(arrows);* spherules *(arrowheads)* can be seen in aquiferous canal, H & E stain; *6,* a group of acidophilic spherulous cells *(arrow)* in ectosome near exopinacoderm; H & E stain; *7,* neutrophilic spherulous cells, H & E stain; *8,* a mixed spherulous cell *(arrow)* on endopinacoderm, trichrome stain. *AC,* aquiferous canal; *C,* choanocyte chamber; *N,* endopinacoderm; *S,* spicule fragments; *SS,* subdermal space; *X,* exopinacoderm.

Table 1. Cell types in Callyspongia (+ = PAS probably positive, + + = PAS distinctly positive, ND = not determined, ? = not observed)

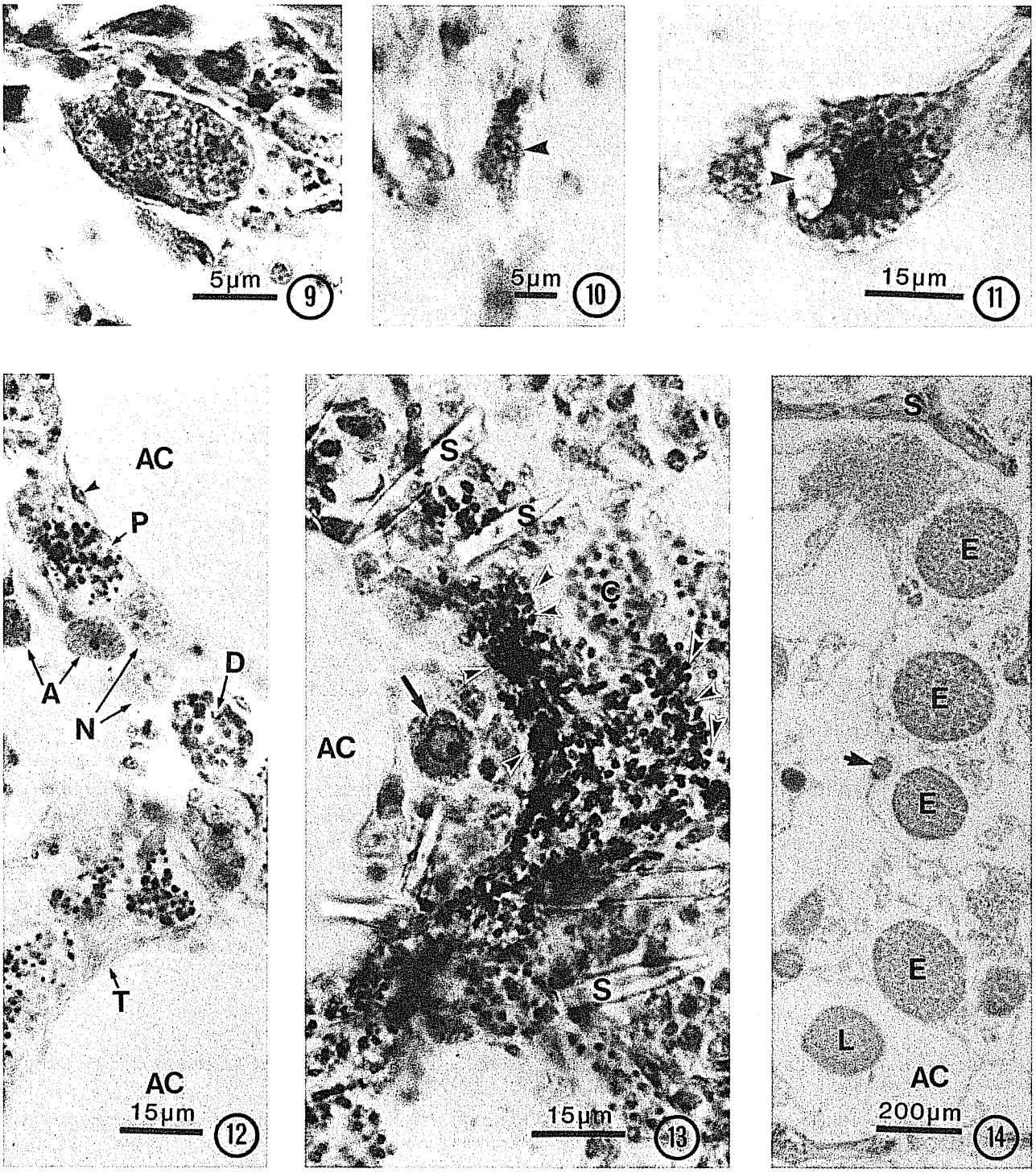
Cell type	Histological staining properties				Nuclear morphology	Cytoplasmic inclusions	Anatomical location	Dimensions (μm, ±SD)	
	H & E	Trichrome	Alcian blue/fast red	PAS				Nucleus	Cell (lxw)
Pinacocytes	pale blue	pale pink	moderately blue nucleolate, central	+	small, lines canals	none	covers surface,	2.02 (± 1.33)	20 (diam.)[a]
Choanocyte	pale blue to clear	pale pink	blue green	+ +	condensed, basal	none	chambers in the choanosome	ND	2.93 × 2.93
Archeocyte	pale blue to dark blue	pink to red	moderately blue central	+	large, nucleolate,	few to many	mesohyl	3.29	15.50 × 4.35 (± 0.66)
Acidophilic spherulous cell	red spherules	red spherules	lavender blue	+	condensed, anucleolate, central	spherules	mesohyl	ND	21.43 × 5.95 (± 7.12)(± 1.84)
Neutrophilic spherulous cell	clear spherules	blue spherules	lavender blue	+	condensed, anucleolate, central	spherules	mesohyl	ND	14.74 × 5.40 (± 3.49)(± 0.88)
Mixed spherulous cell[b]	red and clear spherules	red and blue spherules	?	+	condensed, anucleolate, central	spherules	mesohyl	ND	ND
AMP + cell	pink	blue	dark blue	+	condensed, anucleolate, central	large vacuoles	mesohyl, endopinacoderm	ND	14.32 × 10.50
Acidophilic granular cell[b]	red granules	?	lavender blue	+	moderate, nucleolate, central	many small inclusions	mesohyl, endopinacoderm	ND	5.69 × 4.79 (± 0.50)(± 0.02)
Globoferous cell[b]	?	blue	?	?	obscured by inclusions	many, large, with unstained area	mesohyl	ND	ND

[a]From Johnston and Hildemann, 1982.
[b]Very few cells observed and measured.

to be synchronous while different stages of sperm development are present in different cysts. The mean size of the sperm cyst tends to increase as the sperm inside mature (Table 3). Fertilization occurs within the female sponge after sperm are released from the male and brought into the female with the incurrent water flow (Figure 13). Eggs and embryos are located in the central core of female sponges within the mesohyl (Figure 14). Eggs differ greatly in size, have cytoplasmic inclusions and large nuclei with prominent nucleoli (Figure 13). Parenchymatous embryos develop within females in the same regions where the eggs are located. They are eventually released as ciliated, free swimming larvae (Figure 14) representing the dispersal stage of the sponge life cycle.

Table 2. Germ cells in *Callyspongia* (+ + = PAS distinctly positive, – = PAS negative, ND = not determined, extremely variable depending on the developmental stage)

Cell type	Histological staining properties				Nuclear morphology	Cytoplasmic inclusions	Anatomical location	Diameter (μm, ±SD)	
	H & E	Trichrome	Alcian blue/fast red	PAS				Nucleus	Cell (l × w)
Sperm	not measurable	not measurable	not measurable	–	very small	none	within cysts in the mesohyl near a canal	See Table 3	
Egg	Cytoplasm: pink Inclusions: pink/blue Nucleus: pink/purple	Cytoplasm: red/purple Inclusions: pink Nucleus: red	Cytoplasm: blue Inclusions: pink/red Nucleus: pink/red	+ +	very large, prominent nucleolus	many inclusions, various sizes	central part of the mesohyl	5.4 (±1.99) (nucleolus = 2.37 [±0.86])	35.4 × 22.2 (±18.7)(±12.04)
Embryo	same as egg	same as egg	same as egg	+ +	mostly nucleolate	yolk	same as egg	ND	ND



Figures 9–14. Histology of *Callyspongia diffusa: 9,* acid mucopolysaccharide positive (AMP+) cell in mesohyl, trichrome stain; *10,* acidophilic granular cell *(arrowhead)* on endopinacoderm, H & E stain; *11,* globoferous cell with acentric clear area *(arrowhead),* trichrome stain; *12,* sperm cysts in various stages of development (arrowhead indicates endopinacocyte nucleus), H & E stain; *13,* sperm *(arrowheads)* in choanosome of female sponge near egg *(arrow),* H & E stain; *14,* egg *(arrow),* embryos, and larva in choanosome of a female sponge; larva located within aquiferous canal; alcian blue with fast red stain. *A,* acidophilic spherulous cells; *AC,* aquiferous canals; *C,* choanocyte chamber; *D,* dividing sperm; *E,* embryo; *L,* larva; *N,* neutrophilic spherulous cells; *P,* sperm without tails; *S* skeleton, spicule fragments; *T,* sperm tails.

Table 3. Sperm cysts in *Callyspongia*

Sperm Maturity	Cyst length, μm (± SD)	Cyst width, μm (± SD)
Dividing sperm	18.84 (± 2.16)	13.00 (± 3.66)
Sperm without tails	22.88 (± 3.18)	19.12 (± 2.02)
Sperm with tails	25.84 (± 7.76)	17.34 (± 5.20)

Discussion

The general organization of the typical sponge body consists of choanocyte chambers located at the interfaces of incurrent and excurrent streams of water that pass through a more or less well developed canal system. The unique structure of the choanocyte, with a single flagellum surrounded by a circle of microvilli, or collar, is remarkably constant from one species to another and is a hallmark of the poriferan phylum (Simpson, 1984). Yet, there are some basic anatomical variations within the leuconoid Demospongia. Orientations of the choanocyte chambers differ, implying different strategies of food capture for different species. For example, the choanocytes in *Callyspongia diffusa* and *Reniera* sp. (Langenbruch, 1983) are connected by short basal protrusions (Johnston and Hildemann, 1982) and form spherical chambers that "hang" in the lacunar (incurrent) spaces (Simpson, 1984). Here the choanocytes are the primary cells involved in particle capture and phagocytosis before passing the nutrients to the mesohyl cells for digestion. On the other hand, in a related species *(Ephydatia fluviatilis)* the flow of water passes from the incurrent canal into the mesohyl, where particles are phagocytosed directly by the mesohyl cells before entering the choanocyte chamber (Weissenfels, 1976). Thus, the long evolutionary history of the poriferans has allowed ample time for many successful structural "experiments" based on a flagellated-cell-powered filter feeding organization (Bergquist, 1978).

This long evolutionary history is also reflected in the great diversity of sponge cell morphology and function. For example, all pinacocytes function as epithelial cells in sponges, but in different species they can have quite different morphologies. In *Microciona prolifera,* the exopinacocyte morphology shows an external glycocalyx and a "T" configuration where the nucleus is located in the club shaped cell body that extends into the dermal mesohyl. The endopinacocytes in this species are uncoated and fusiform (Bagby, 1970). In *Callyspongia diffusa,* however, all pinacocytes are uncoated, fusiform, and identical to each other. Conversely, archeocytes in different sponge species have a similar morphology, but they may be quite diverse in function. Variability in the sizes and number of cytoplasmic inclusions in the archeocytes of *C. diffusa* seems to indicate only different stages in the digestion of phagocytosed food particles, but other possibilities for archeocyte function can be inferred from this study. *Callyspongia diffusa* secretes silicious spicules and encases them in spongin, and in some situations, such as graft rejection and inflammation (Smith and Hildemann, 1986a) will secrete small amounts of sponge collagen. Such activities suggest the presence of sclerocytes, spongocytes, lophocytes or collencytes, but these cell types have not been identified in this species by their defined morphological characteristics (see Simpson, 1984). The possibility remains that the archeocytes in *C. diffusa* perform all of these functions. This has been suggested previously from studies on *M. prolifera,* where sclerocytes appear morphologically identical to archeocytes (Simpson, 1978).

In general, the activities of archeocytes in *Callyspongia diffusa* may include: (a) secretion of the skeleton, (b) phagocytosis of food particles and injury induced cell debris (Smith and Hildemann, 1986a), and (c) regeneration of damaged or lost cells (Smith and Hildemann, 1986a). In cytotoxic reactions to allogeneic contact, only a subpopulation of archeocytes seems to be directly involved at the graft interface (Smith and Hildemann, 1986b). This suggests that the archeocytes in *C. diffusa* are not a homogeneous population of cells capable of performing all types of functions, but that they may be a conglomerate of functionally differentiated subpopulations that includes a pluripotential stem cell.

The remaining mesohyl cells are an assemblage of diverse, poorly characterized, and confusing cell types (Simpson, 1984). The variety of interpretations of previous morphological investigations of sponge cells has added to the confusion already imposed by existing sponge diversity. In many instances, analyses have not progressed beyond simple cellular identifications. Such problems can be illustrated with several of the mesohyl cells in *Callyspongia diffusa.* There is a disagreement on the identification of acidophilic spherulous cells in another *Callyspongia* species. Pomponi (1976) calls this cell type a granular cell. The mixed spherulous cells may be a distinct cell type, but they may also be transitional forms between the acidophilic and neutrophilic spherulous cells, or remnants of acidophilic spherulous cells after spherule release. The identification of AMP+ cells is not certain. In some aspects, these cells are morphologically similar to AMP+ cells identified by Simpson (1963, 1968), and in others, they are similar to spongocytes characterized by Garrone and Pottu (1973). The acidophilic granular cells are also confusing. They seem most similar to eosinophilic amoebocytes in calcareous sponges (Simpson, 1984), and they also resemble some descriptions of gray cells (Boury-Esnault and Doumenc, 1979) although eosinophilia is not

typical of most gray cells (Boury-Esnault, 1977). Final identification of these cell types will have to await more detailed analysis of cells from many sponge species.

Functional considerations of the less common mesohyl cells are not numerous. However, interest in the spherulous cells has yielded some interesting speculations. A nonspecific defense role for acidophilic spherulous cells in *Callyspongia diffusa* is suggested from the location of groups of these cells close to the surface, near incurrent subdermal spaces (Figure 6). In addition, their mass release of spherules into the canal system (Figure 5) may have a protective function. These speculations are supported by an antibiotic activity of spherulous cell contents from other marine sponges (Thompson et al., 1983). Alternatively, spherulous cells may also be involved in spongin secretion. Neutrophilic spherulous cells in *C. diffusa* stain blue with Masson's trichrome stain, as do the skeletal spongin and small patches of mesohyl collagen. These cells may have spongin secreting activity, even though they do not appear to be associated with the spongin casing around the skeleton nor with the collagen deposits as has been observed for spongocytes and spherulous cells in other species (Garrone and Pottu, 1973; Bretting et al., 1983). It is noteworthy that these two activities, nonspecific defense and spongin secretion, are found in morphologically similar spherulous cells in *Axinella polypoides* (Bretting et al., 1983), whereas, in *C. diffusa* these activities may be localized in different types of spherulous cells.

Conclusions

Future studies of sponge cell biology may show that a variety of morphologically different cells in different species perform similar functions and, conversely, that morphologically similar cells can perform very different functions even in the same species. In light of the age of this phylum and the various successful adaptations by sponges to challenges presented by different environments, final and definitive characterizations of sponge cell types may eventually have to be based primarily on the functions that the cells perform with secondary importance given to cellular morphology and anatomical locations.

Acknowledgments

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