Proc. R. Soc. Lond. B **226**, 445–464 (1986)
Printed in Great Britain

Allograft rejection, autograft fusion and inflammatory responses to injury in *Callyspongia diffusa* (Porifera; Demospongia)

By L. C. Smith† and W. H. Hildemann‡
Department of Microbiology and Immunology, School of Medicine, University of California, Los Angeles 90024, *U.S.A.*

(*Communicated by D. C. Smith, Sec.R.S. – Received* 13 *June* 1985)

[Plates 1–6]

Sponges exhibit a variety of swift, cellular defence responses to protect self integrity. The sponge *Callyspongia diffusa* has been used to characterize the cytological changes that occur during allograft rejection, autograft fusion, and inflammation. Allogeneic contact results in fusion of the two exopinacoderms followed by an infiltration of mesohyl cells into the graft zone. As mesohyl cells accumulate, they form tissue bridges that span the graft interface. After a few days, the tissue bridges and the nearby cellular infiltrate become necrotic and slough off, which separates the allogeneic tissues.

Autograft fusion begins similarly but cellular infiltration does not follow exopinacoderm fusion. Contacted exopinacocytes are redistributed, the endopinacoderms and choanosomes come into contact, and the grafted sponge tissues merge.

Tissue damage exposes internal regions of the sponge to the external environment. In many areas of injury, exposed choanosome is sealed by infiltrating mesohyl cells. In other areas, exposed endopinacoderm appears to serve as new exopinacoderm. Cellular debris is removed by phagocytic archaeocytes and new exopinacoderm is regenerated over the damaged choanosome. No scars remain once the inflammatory infiltrate has dispersed.

In general, mesohyl cells are involved in defence responses without an observed enrichment of any specific cell type. However, archaeocytes from rejecting sponges appear to line both sides of the allogeneic interface.

Introduction

The survival of multicellular organisms depends, in part, on abilities to recognize and reject encroaching or invading 'non-self' cells and tissues, and to repair tissue damage. Damage and fragmentation occur frequently in sedentary, aquatic

† Present address: Division of Biology, California Institute of Technology, Pasadena, California 91125, U.S.A.

‡ 1927–1983.

organisms such as sponges as a result of both biological and physical environmental effects. Predators and parasites damage sponges by feeding upon them (Randal & Hartman 1968; Resh 1976). Fragmentation from storm-induced wave action has been suggested as one mechanism of tissue damage (Jokiel *et al.* 1982), although some species fragment to reproduce asexually (Neigel & Avise 1983; Neigel & Schmahl 1984), and others fragment as a consequence of seasonal changes (Stone 1970). Sponges are known for their regenerative capabilities from classical reaggregation studies of dissociated cells (Wilson 1907; Galtsoff 1925; Humphreys 1963) and from inflammatory responses experimentally induced by injury (Jones 1957; Korotkova 1962, 1970).

Various non-self contacts challenge the integrity of sponges including invasion by microbial pathogens, metazoan parasites and natural metazoan tissue transplantations. Because sponges have exposed surface cells, direct cellular contact from other sedentary metazoans (including other sponges) is a consequence of nearby larval settlement and normal growth in crowded habitats. To assess general sponge immunity, comparative immunologists have capitalized upon the phenomenon of natural tissue transplantation and have found that sponges can reject experimentally transplanted tissues. Little information is available on natural challenges to self integrity such as microbial and parasitic defences (for review, see Johnston & Hildemann 1982), yet sponges may use defence mechanisms similar to those exhibited in experimental transplantation rejections (Smith & Hildemann 1984).

Sponge alloimmune responses to experimental transplants show two major rejection processes. Some species construct barriers to separate or wall off non-self tissues (Van de Vyver 1980; Kaye & Ortiz 1981; Buscema & Van de Vyver 1983, 1984*b*; Neigel & Avise 1983; Neigel & Schmahl 1984), while others mount a cytotoxic response against allogeneic cell contact (Hildemann *et al.* 1979, 1980; Evans *et al.* 1980; Hildemann & Linthicum 1981; Bigger *et al.* 1981, 1982, 1983; Van de Vyver & Barbieux 1983; Buscema & Van de Vyver 1984*a*). A few species show responses to non-self that do not strictly correspond to either of these two mechanisms (Paris 1961; Neigel & Avise 1983; Smith & Hildemann 1984).

Callyspongia diffusa, a ramose, Indo-Pacific sponge, exhibits a cytotoxic response to allografted tissues with short-term alloimmune memory (Hildemann *et al.* 1979, 1980; Bigger *et al.* 1982). The parabionts respond to direct and continued allogeneic contact with mesohyl cell infiltration that forms tissue bridges (structurally weak extensions of sponge mesohyl tissue that span the graft zone to contact the grafted tissues) and ends with uni- or bilateral necrosis that results in allogeneic tissue separation (Hildemann *et al.* 1980; Johnston & Hildemann 1983). Rejection times range from 4.5 to 15.5 days depending on sponge pairings and water temperature (Johnston *et al.* 1981).

In initial efforts to define cytological changes that correspond to macroscopic observations of graft rejection, Johnston & Hildemann (1983) observed archaeocytes and unidentified vacuolated mesohyl cells migrating into the graft zone. As these cells accumulated they obliterated local subdermal spaces and aquiferous canals, they squeezed or pushed choanocyte chambers out of the area and formed tissue bridges.

The scanning electron microscopic technique used by Johnston & Hildemann

(1983) made it difficult to identify many of the mesohyl cell types and to determine confidently the cellular composition in the graft zone of *C. diffusa*. To clarify these points, routine histological procedures have been used to identify cell types (Smith 1985), to analyse the rejection process and to attempt an identification of the sponge 'immunocyte'. Allograft rejections (immunospecific responses) were compared with autograft fusions (responses to self contact) and with injury-induced inflammation (non-specific defence reactions). Results show active migrations of mesohyl cells towards both rejection zones and areas of tissue damage. Differential cell counts of allo- and autograft zones and areas of injury indicate no differences in mesohyl cell composition. However, archaeocytes form 'fronts' at allograft interfaces suggesting that a specific cellular interaction may occur during responses to allogeneic contact.

Materials and methods

Sponges

Callyspongia diffusa is a brilliant purple sponge found on reef crests in Kaneohe Bay, Oahu, Hawaii. Several large specimens were collected from around Coconut Island and from the Kaneohe Yacht Club patch reef and maintained in unfiltered, running seawater at the Hawaii Institute of Marine Biology (University of Hawaii). Some sponges were sent from Hawaii to Los Angeles by air express where they were kept at U.C.L.A. in a 20 gallon (*ca.* 75 l) seawater aquarium equipped with a heater, a 'Gro' lamp (Penn-Plax, Inc.), and a power filter. Sponges were fed 3 ml of commercially available invertebrate liquid filter feeder food twice daily, and a suspension of commercially available unicellular algae was added slowly and continuously via a flow regulator from an intravenous fluid bag. In all experiments, the animals were used within four days of collection.

Grafting procedures and injury induction

Sponges were grafted by the methods developed by Johnston & Hildemann (1983). Small finger-like growths of sponges were cut into short pieces of 3–5 cm and allowed to heal for 24–48 h. Pairs of sponge pieces were secured to labelled plastic splints (1 inch by 3 inch, *ca.* 2.4 cm by 7.2 cm) with nylon fishing line. Eight allografts were paired in replicates for analysis at various time periods, ranging from 1 to 120 h (1, 3, 6, 12, 16, 24, 48, 72, 96, 120). As controls, seven autografts were paired in replicates for analysis at 1–48 h (1, 3, 6, 12, 24, 48). In addition, non-specific defence reactions to injuries were used as controls for comparison to immunospecific allorejection responses. Finger-like extensions of three sponges were cut cross-wise with dissecting scissors. The resulting 3 cm pieces were allowed to heal for analysis at 0.5–48 h (0.5, 1, 2, 4, 6, 8, 12, 24, 48).

Histological procedures

Sponges were processed for histological analysis according to the methods of Smith (1985). Sponge fragments and grafts were immersed for 12–16 h in Bouin's fixative, rinsed in seawater and tap water, dehydrated in ethanol and xylene and embedded in paraplast (Monoject Scientific). When the blocks were cooled to

−25 °C in a cryostat, the paraplast was hardened enough to obtain sections of tissue that contained skeletal spicules. Tissue orientation was checked on unprocessed sections by staining with 1 % (by mass) toluidine blue. Those chosen for analysis were routinely stained with Harris's haematoxylin and eosin (H & E), periodic acid–Schiff (PAS), alcian blue with fast red as a counter stain, and Masson's trichrome stain. Sections were observed and photographed on Plus X black and white film with a Zeiss or Olympus photomicroscope. To identify mitotic figures, DNA was stained with 4,6-diamidino-2-phenylindole (DAPI) 2 mg ml^{-1} (Sigma) and observed in a Leitz Ortholux fluorescent microscope.

Results

Normal anatomy

The microanatomy and cytology of *C. diffusa* was typical for a leuconoid demosponge. It was composed of choanocyte chambers, aquiferous canals and mesohyl (figure 1, plate 1) (Simpson 1984; Smith 1985). Flagellated choanocytes formed the spherical choanocyte chambers or water pumps. The aquiferous canal system, lined by endopinacoderm (endothelial-like cells) served to transport seawater to and from the choanocyte chambers for filter feeding. The mesohyl (the true internal regions of the sponge body) was composed of seven cell types (Smith 1985). Those mentioned within this paper include archaeocytes (amoeboid phagocytes), neutrophilic and acidophilic spherulous cells, and acid mucopolysaccharide positive (AMP+) cells. The exopinacoderm (or sponge epidermis), which was raised into numerous points by the underlying and sometimes protruding skeleton, resulted in a surface view that looked like the top of a circus tent.

Allograft rejection

When two sponges were placed into contact, the spicules protruding from the surface punctured the opposing expinacoderm. These surface layers fused at these puncture points (figure 2, plate 2), after which the fusion spread throughout the entire graft zone (figure 3). By 3 h, the mesohyl cells, normally located just under the exopinacoderm at the points where the spicules protruded from the surface, began to accumulate around the fused exopinacoderms.

The mesohyl cells migrated into the graft zone from the surrounding mesohyl. Initially, this occurred as a generalized cell migration through the mesohyl, but as the rejection process continued, the cells began to travel in mesohyl tracts that were oriented perpendicularly or obliquely to the graft interface (figure 4). Accumulations of the mesohyl cells obliterated the fused exopinacoderms; consequently the fate of this structure could not be followed. Between 6 and 48 h, the local aquiferous canals were 'filled in' or rerouted and local choanocyte chambers were squeezed out of the area by the accumulating mesohyl cells (figure 5).

In the few places where the cellular alignment and plane of section corresponded, the exact allogeneic cell contact interface could be located. In these areas, the allogeneic cells lined up against each other in 'fronts' (figures 6–8, plate 3) (see Simpson 1984). Moreover, these cellular fronts occurred where tissue bridges

contacted allogeneic tissues (figure 9). The front row of cells at the allogeneic interface appeared to be composed of archaeocytes (figures 9 and 10). Small deposits of sponge collagen, identified by the alcian blue and trichrome stains, were located within regions of cell accumulations and just to one side or the other of the graft interface (figures 4 and 7). Archaeocyte phagocytosis of cell debris was not discernible. In one specimen at 24 h, areas of parabiont separation had begun at the original line of exopinacoderm contact. However, points of allogeneic contact remained in this specimen where small tissue bridges were present.

There were substantial increases in numbers of cells that accumulated in the graft zone between 48 and 96 h. Large open spaces appeared within these patches of cells, along with a concurrent formation of tissue bridges that maintained allogeneic contact by spanning the graft interface (figure 10). Large mesohyl tracts, impinging upon the graft interface, appeared to supply cells to these large tissue bridges. Small isolated areas of collagen deposition were present (between 48 and 96 h) within the infiltrated mesohyl cells just on either side of the line of allogeneic contact.

Unilateral killing and invasion in allograft responses were observed in this study. In a unilateral response, large tissue bridges from one sponge of the pair invaded the vacated skeleton of the other (figure 11, plate 4). Whether this vacated, exposed skeleton was a result of tissue retreat or cellular necrosis was not discernible in the specimen illustrated. The tissues of the 'losing' or 'retreating' sponge in the lower half of figure 11 appeared to maintain an exopinacoderm.

Between 72 and 120 h, large tissue bridges and areas of mesohyl cell accumulations located behind the bridges became necrotic, broke down and were washed away by the environment (or the tissue processing) leaving a large separation gap between the parabionts. Once necrosis was initiated, tissue bridges became very fragile owing to lack of structural support. Break down and sloughing happened quickly and fixation did not strengthen these necrosing structures enough to withstand processing. Therefore, only pre- and post-separation allografts were presented. After tissue separation, the retreating sponge in a unilateral response (figure 12, top) appeared moribund with no exopinacoderm. Only a rim of tissue containing functional choanocyte chambers were observed near the top of the figure, the rest was composed of patches of condensed mesohyl cells and necrotic tissue. The other sponge of this pair (figure 12, bottom) was left with only two thirds of its original tissue after the disintegration of large tissue bridges and adjacent regions of accumulated mesohyl cells. Cellular degradation exposed the skeleton and left the edge of the remaining tissues ragged with remnants of infiltrated mesohyl cells and tissue bridges. Small collagen deposits were still present within these areas.

The skeleton, which was resistant to disintegration because of the spongin casing around the spicules, revealed the original configuration of the sponge pair (figures 11 and 12). Formation of new exopinacoderm over the remaining tissues completed the rejection process, however, this final step was not observed in any of the specimens prepared for this study and the origin, or origins, of new exopinacocytes remained undetermined.

The cells found in graft zones included archaeocytes, acidophilic spherulous cells,

neutrophilic spherulous cells, and AMP+ cells. Differential cell counts of the cells accumulated at the graft interface at 1, 12, 24, 48, and 96 h showed relative cell compositions to be 91% archaeocytes, 4.7% acidophilic spherulous cells, 5% neutrophilic spherulous cells and under 1% AMP+ cells. These data were not significantly different from the composition of normal mesohyl in *C. diffusa* (Smith 1985) although there was a slight increase in the percentage of archaeocytes and a corresponding decrease in the spherulous cells present in the graft zone. This slight increase in archaeocytes was due to this cell type lining up at the allogeneic interfaces (see figures 6–9).

The term hyperplasia has been used to describe the gross morphological changes that occur at the rejection zones and is defined as a numerical increase in cells resulting from mitoses. To determine if mesohyl cell mitoses contribute to the accumulations of cells at the graft interface, a series of allograft sections (corresponding to specimen number 3, figure 22), ranging from 1 to 24 h were stained with DAPI. When observed in a fluorescent microscope, no mitotic figures were noted either at the graft interface or in the general tissues of the sponges (data not shown).

Autograft fusion

In investigations of sponge alloimmunity, autografts have served as controls to indicate the ability of sponges to differentiate between self and non-self. Details

---

Description of plate 2

Figure 2. Allograft. An early parabiosis in which exopinacoderm (X) contact and fusion (arrows) can be seen at points of spicule protrusion. Mesohyl cells are located just below the exopinacoderm at these points. s.s., Subdermal space; n.c., normal choanosome. Trichrome; scale bar, 0.2 mm.

Figure 3. Allograft. Exopinacoderm fusion (arrows) can be seen throughout most of this graft interface. Associated mesohyl cells make this fused structure appear thicker than two normal exopinacoderms (X). n.c., Normal choanosome. Trichrome; scale bar, 0.2 mm.

Figure 4. Allograft. A large mesohyl tract (arrows) oriented towards the graft interface is denoted by ●. The arrow head indicates a collagen deposit. Alcian blue, fast red; scale bar, 0.2 mm.

Figure 5. Allograft. Local choanocyte chambers appear squeezed together by the mesohyl infiltration. s.c., Squeezed choanocyte chambers; c., normal choanocyte chambers; m.a., mesohyl cell accumulation; t., mesohyl tract; s., skeleton; w., cross sections of a parasitic worm. H & E; scale bar, 80 μm.

Description of plate 3

Figure 6. Allograft. A graft zone shows the cellular interface (between the ● symbols). A large mesohyl tract is indicated by the arrow. PAS; scale bar, 0.5 mm.

Figure 7. Allograft. The allogeneic cell interface is denoted by ●. Small deposits of collagen (arrow heads) are located just to one side of the graft interface. H & E; scale bar, 0.1 mm.

Figure 8. Allograft. The allogeneic cell interface is denoted by ●. H & E; scale bar, 30 μm.

Figure 9. Allograft. Allogeneic cells are in contact (between the ● symbols) at the end of the tissue bridge. Alcian blue, fast red; scale bar, 20 μm.

Figure 10. Allograft. Large tissue bridges (b.), with open spaces (o.) between them, span the graft zone. The arrow indicates a mesohyl tract. H & E; scale bar, 0.5 mm.

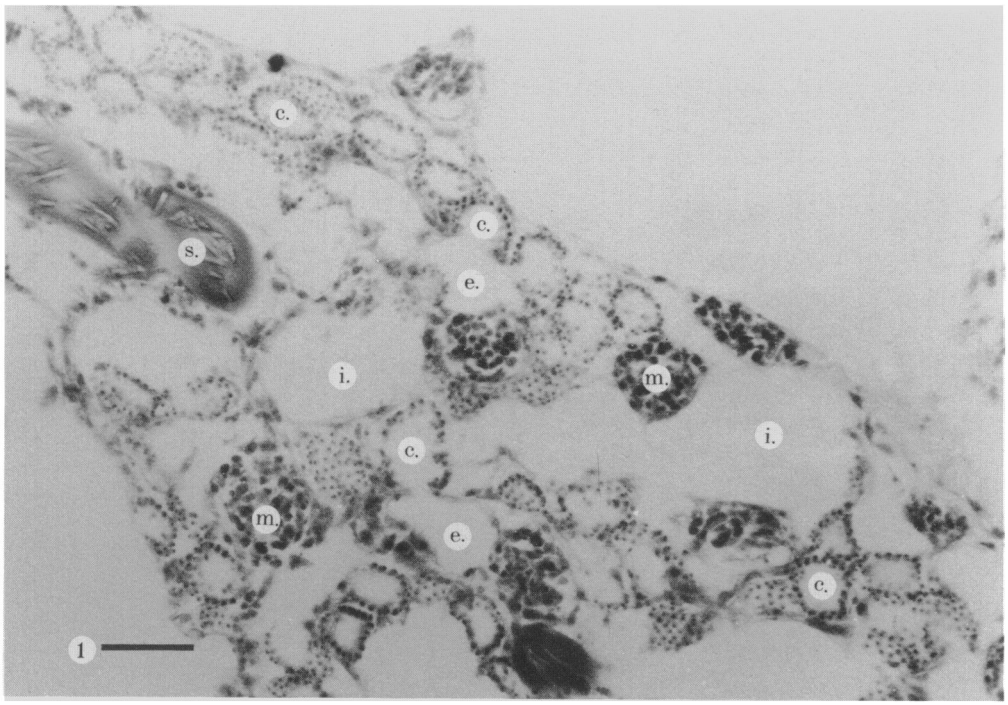
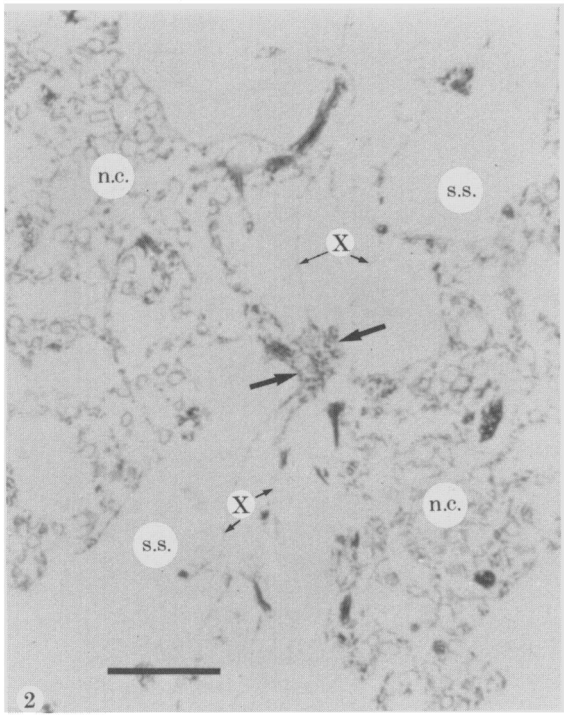
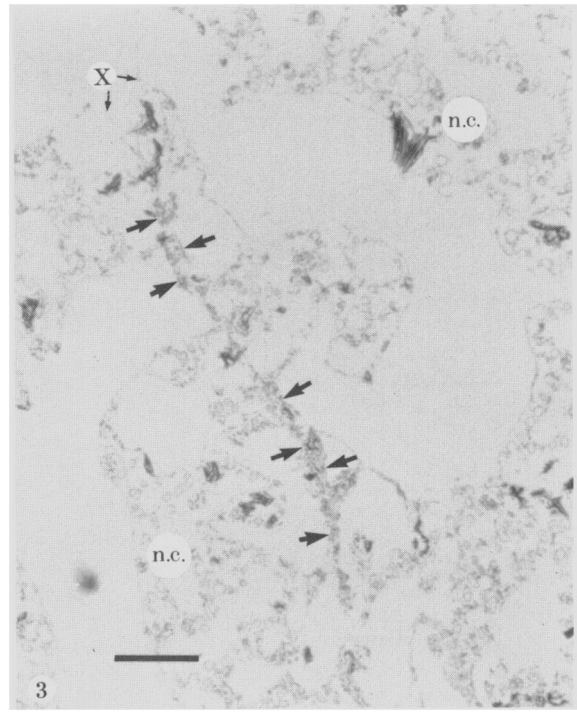
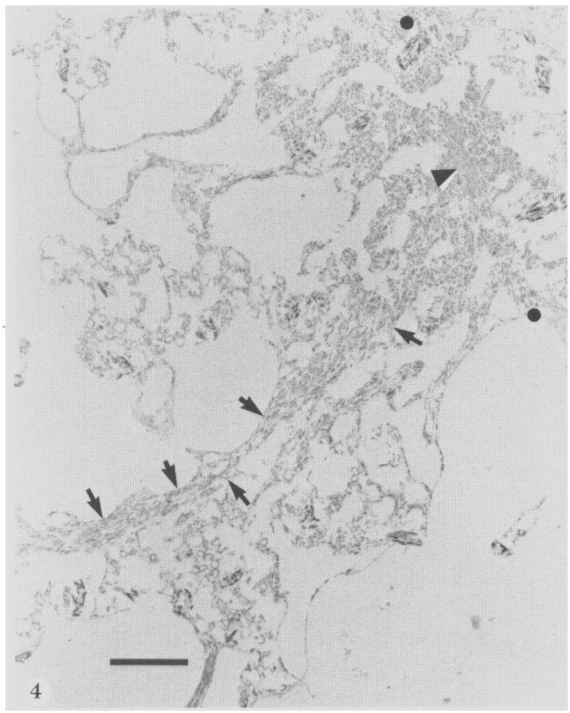


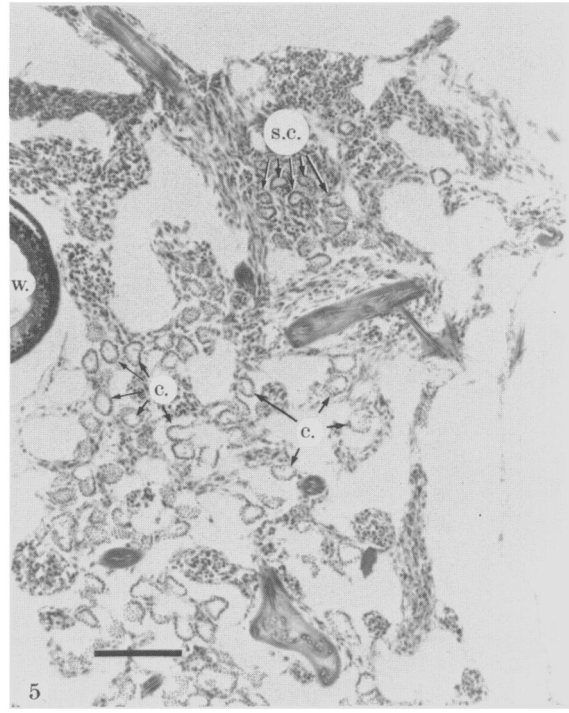
FIGURE 1. Normal choanosome. c., Choanocyte chamber; m., mesohyl; i., incurrent canal; e., excurrent canal; s., skeleton. H & E; scale bar, 30 μm.

(*Facing p.* 450)

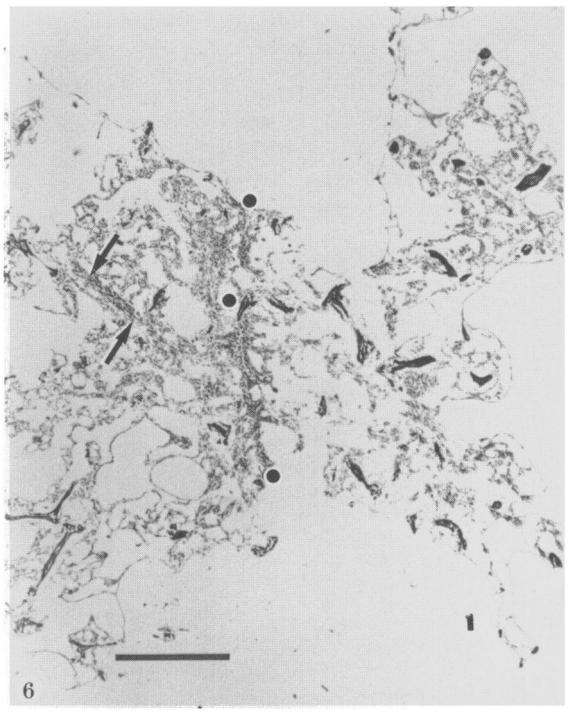


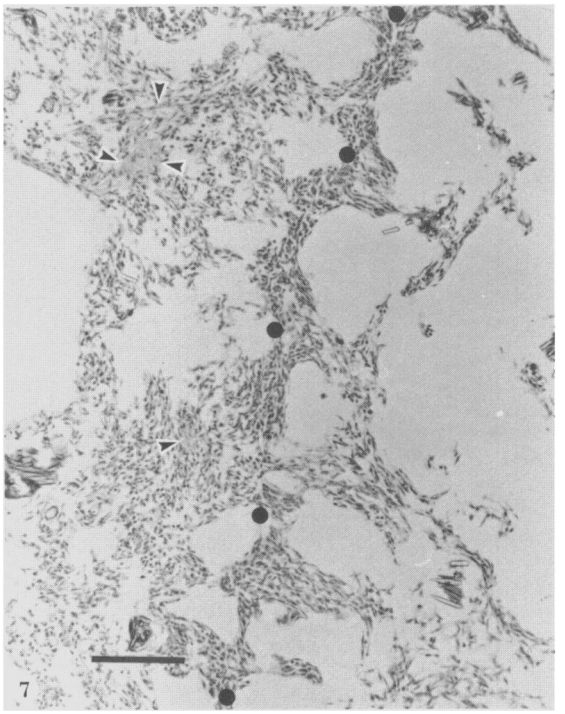


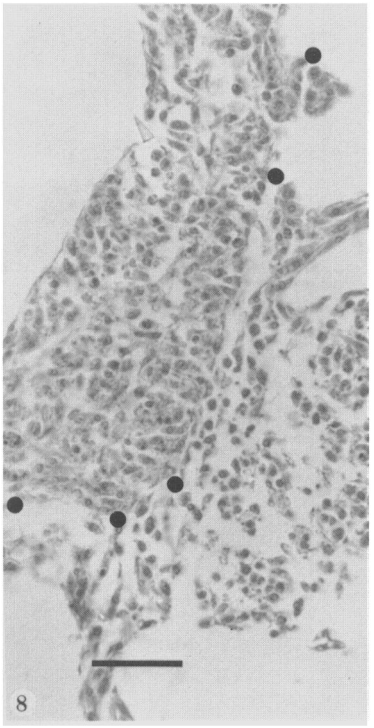


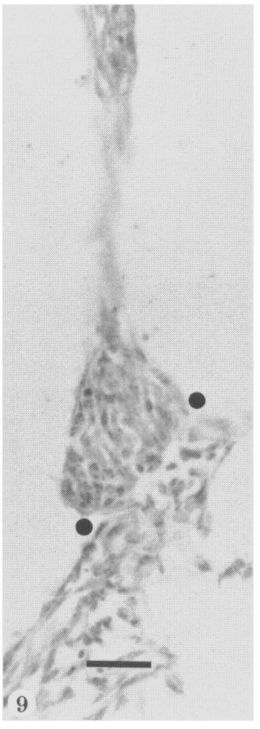


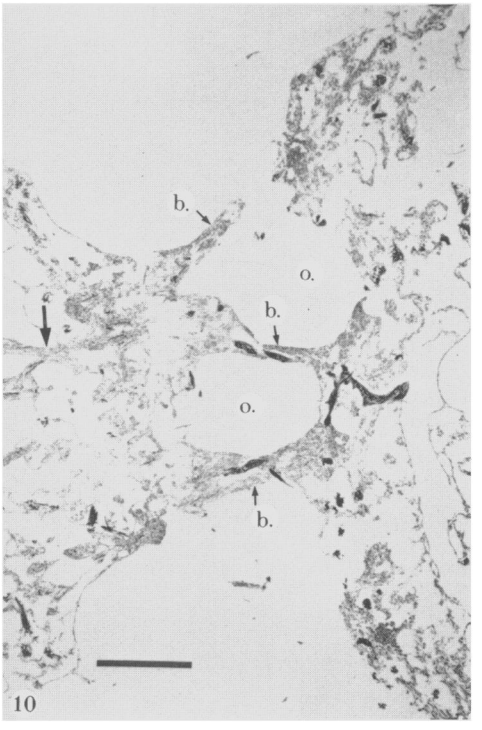
FIGURES 2–5. For description see p. 450.



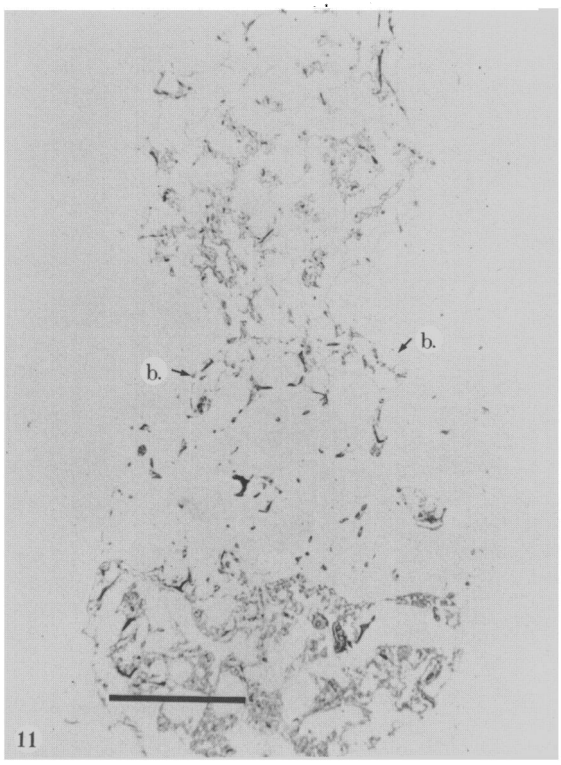


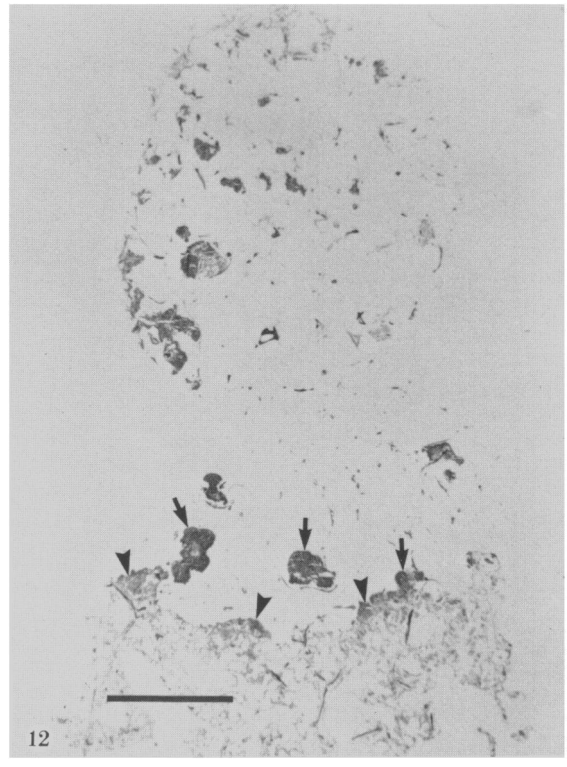


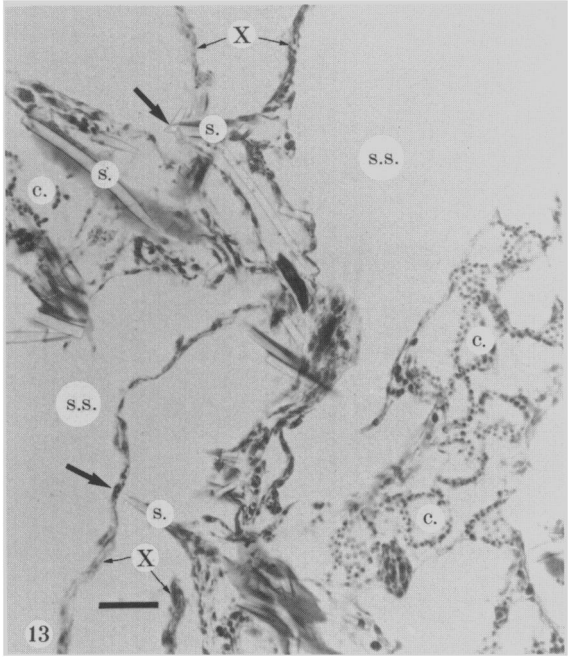


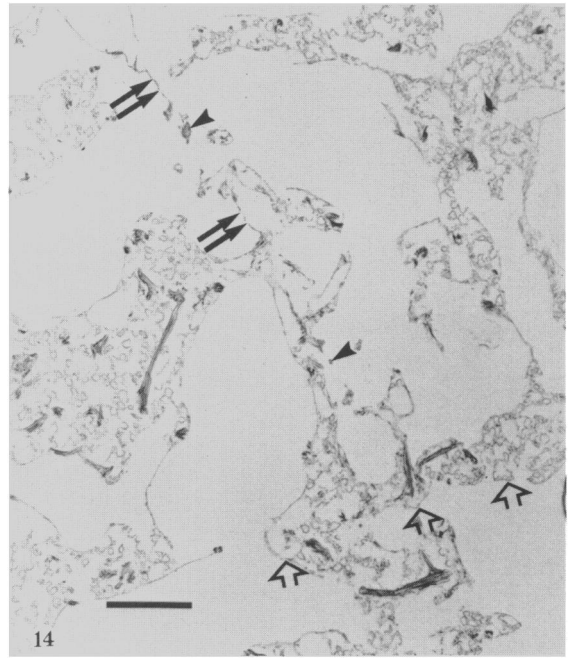


FIGURES 6–10. For description see p. 450.

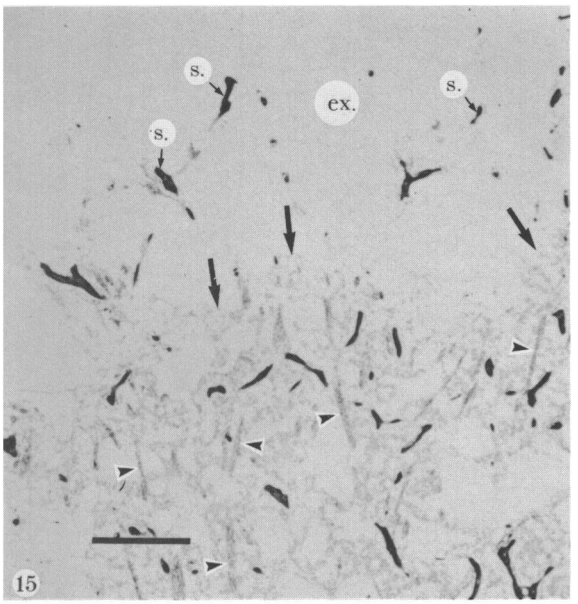


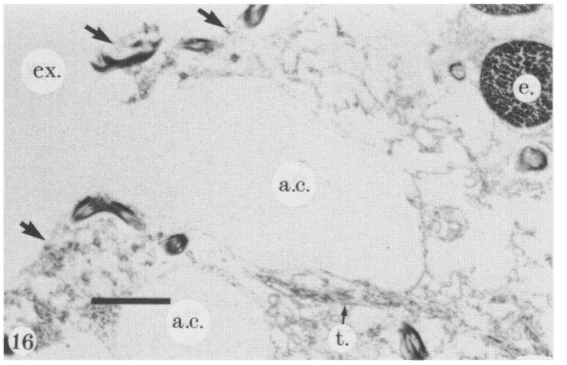


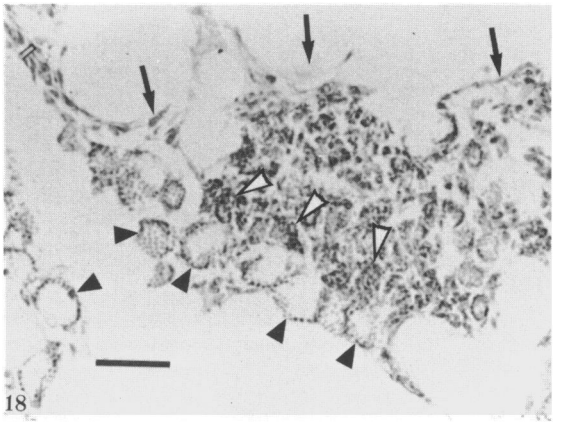


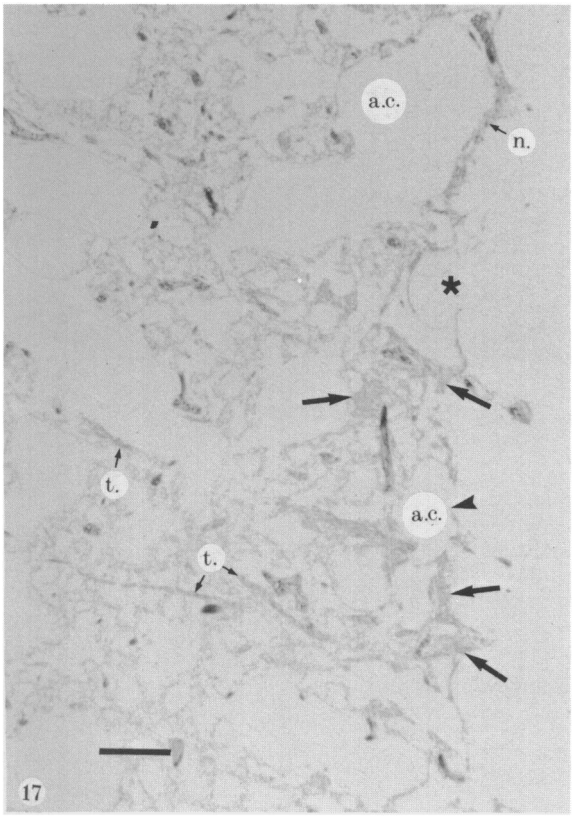


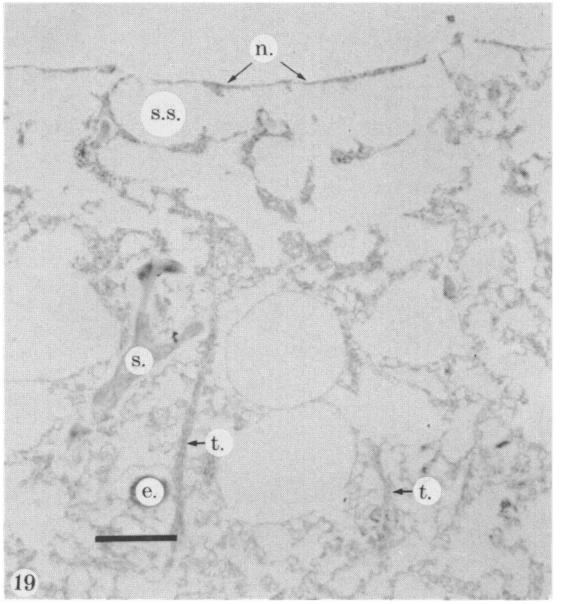
FIGURES 11–14. For description see p. 451.











FIGURES 15–19. For description see p. 451.

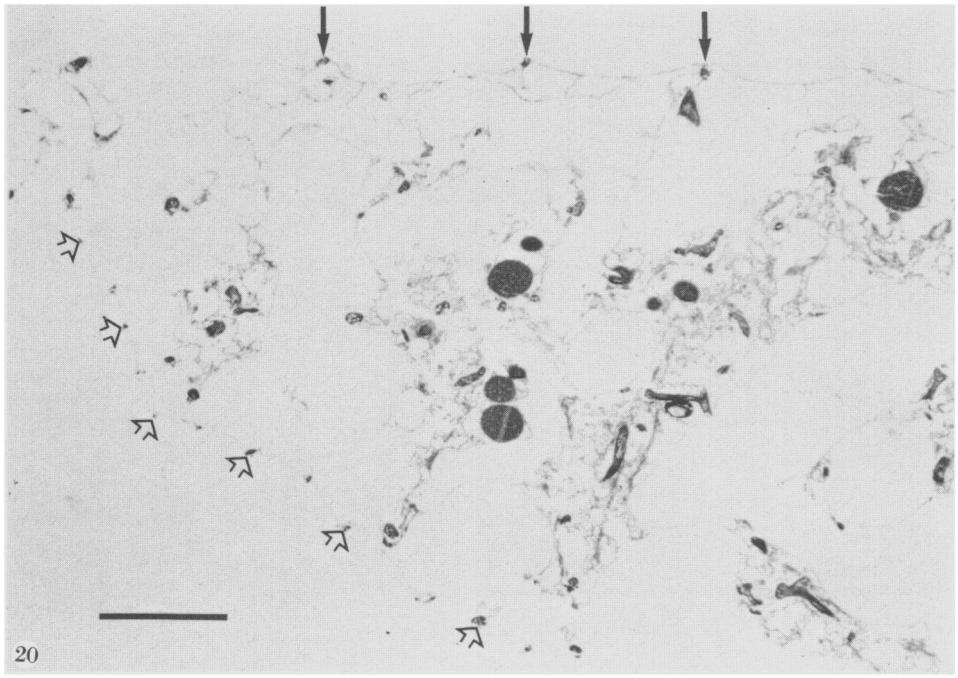


FIGURE 20. Inflammation. No scars can be seen after healing is completed. Fewer spicular projections result in a flatter appearance of the healed edge (solid arrows) compared with the numerous spicules on the control, uninjured edge (open arrows). Morphological differences can be seen in the subdermal spaces located below the healed versus the uninjured exopinacoderms. H & E; scale bar, 0.5 mm.

of the cellular interactions that occurred during autograft fusions were different from those seen in allograft rejections.

As was noted for allografts, when sponges were autografted the points of the prot uding spicules punctured the opposing exopinacoderm (figure 13). The excpinacoderms fused at these contact points and within 3 h the fusion spread throughout the graft interface.

By 6 h the fused exopinacoderms began to break up into isolated 'dots' of pinacocytes located in the original line of the graft interface. As the exopinacocytes were redistributed, the endopinacocytes that lined the canals in the area came into contact, and by 12 h the two aquiferous canal systems had begun to join. As the exopinacoderms continued to break up, the endopinacocytes and the canal system

---

Description of plate 4

Figure 11. Allograft. Tissue bridges (b.) extend into the vacated skeleton of the paired sponge. Original positions of the two sponges can be determined from the location of the skeletons. PAS; scale bar, 2.0 mm.

Figure 12. Allograft. Necrosis of the tissue bridges separates the allografted sponges and leaves remnants of tissue bridges (arrows) and areas of cellular infiltrate (arrow heads). Most of the tissue in the sponge in the upper half of the figure has degenerated and is unorganized except for a thin rim of tissue at the top of the figure that contains choanocyte chambers. The sponge in the lower half of the illustration has had about half of its tissues destroyed. The original orientations of the grafted pair can be determined from the locations of the skeletons. Trichrome; scale bar, 2.0 mm.

Figure 13. Autograft. This parabiosis has resulted in the puncture of the exopinacoderms (arrows) by the opposing spicules. The exopinacocytes and mesohyl cells are in contact. X, exopinacoderm; s., spicule; s.s., subdermal space; c., choanocyte chamber. Trichrome; scale bar, 25 μm.

Figure 14. Autograft. The fused exopinacoderms (double arrows) appear to break up into 'dots' (arrow heads). The choanosomes are fused in some areas (open arrows). Alcian blue, fast red; scale bar, 0.25 mm.

Description of plate 5

Figure 15. Inflammation. Newly injured tissue appears ragged and open to the exterior. An exopinacoderm is absent (arrows) and bare skeleton (s.) protrudes. Tracts of mesohyl cells (arrow heads) are oriented towards the injury. ex., Exterior. Trichrome; scale bar, 0.5 mm.

Figure 16. Inflammation. A large aquiferous canal (a.c.) has been opened to the exterior (ex.) by the injury. The arrows indicate the injured edge. t., Mesohyl tract; e., embryo. H & E; scale bar, 0.15 mm.

Figure 17. Inflammation. The injured edge appears to be sealed by two methods: (i) the endopinacoderm lining the large aquiferous canals (a.c.) serves as new exopinacoderm (arrow head), and (ii) cells, which infiltrate via mesohyl cell tracts (t.), accumulate and occlude the damaged choanosome (arrows). These infiltrating cells form a thick 'neo-exopinacoderm' (n.) which seals large aquiferous canals. H & E; scale bar, 30 μm.

Figure 18. Inflammation. Damaged choanocyte chambers and single choanocytes can be seen at the injured edge (arrows). White arrow heads indicate the damaged choanocytes and choanocyte chambers, whereas black arrow heads show normal choanocyte chambers. Alcian blue, fast red; scale bar, 50 μm.

Figure 19. Inflammation. Thick neo-exopinacoderm (n.) covers partly reconstructed subdermal spaces (s.s.). t., Mesohyl tracts; s., skeleton; e., embryo. H & E; scale bar, 0.2 mm.

increased their connection, and by 24 h, the choanosomes (the deeper portions of the sponge) came into contact and also began to join together (figure 14).

Choanosome fusion continued until it was no longer possible to locate the original graft zone. By 48 h, only the external shape of the fused sponges such as an indentation of the exopinacoderm or an abrupt change of external configuration due to a grafting misalignment indicated the original region of contact. Skeletal fusion also occurred during autografting but this aspect of graft acceptance was not discernible with the techniques used in this study.

Inflammatory response to injury

It was of interest to investigate other types of cytological responses in sponges. In addition to rejection of transplanted tissues and defence against microbial pathogens, sponges also respond to tissue damage. An analysis of the inflammatory response to injury in *C. diffusa* allowed a direct comparison of a non-specific defence reaction to that of a specific alloimmune rejection reaction.

When a branch of *C. diffusa* was cut through the ectosome and choanosome, tissues and skeleton were compressed. Immediately after cutting, the spongin that encased the skeleton caused the sponge fragment to snap back to its original shape and a cloud of cells escaped through the injury into the environment. When sectioned, this injured edge appeared ragged and open; it lacked an exopinacoderm, and the choanosome was exposed, large canals were open to the exterior and bare skeleton protruded from the edge of the injury (figures 15 and 16, plate 5). In one specimen, an inflammatory response to the injury was initiated within 30 min as evidenced by a migration of mesohyl cells along tracts to the area of damage (figure 15).

By 1 h, the injured tissues had receded from the open edge somewhat, exposing more of the skeleton. The edge, initially open to the exterior, was sealed within 8 h by two processes. First, where large canals that ran parallel to the injury were exposed, the endopinacoderm lining these canals appeared to serve as a new exopinacoderm (figure 17). Second, the choanosome was occluded by an inflammatory infiltrate that consisted of all the mesohyl cell types, and arrived via mesohyl tracts (figures 15–17, 19). Cellular infiltration and inflammation did not occur in areas sealed by the endopinacoderm. The large aquiferous canals that ran perpendicular to the injury were closed by the formation of a thick 'neo-exopinacoderm' over the openings (figure 17). All mesohyl cell types presumably including the (unidentified) exopinacocyte precursor were present on this structure. Many non-functional choanocyte chambers and abnormal groups of single choanocytes were present at and near the edge of the injury (figure 18) and many of the infiltrated archaeocytes were filled with phagosomes. Phagocytic archaeocytes normally contain phagosomes and whether those near the injury contained more phagosomes than average was not determined. Small patches of collagen deposited within the cell accumulations appeared similar to those noted in allograft responses.

Many mesohyl tracts were oriented towards the healing areas between 8 and 24 h. During this time, mesohyl cells continued to accumulate at the edge and formed distinctive areas of the inflammatory response in the choanosome

(figure 17). Although single choanocytes, non-functional chambers, and phagosome-filled archaeocytes were present in the damaged tissues at 24 h, the cellular infiltration rate began to diminish. This was indicated by (i) fewer mesohyl tracts impinging on the site of injury; (ii) smaller patches of accumulated cells; (iii) fewer non-functional choanocyte chambers and single choanocytes; and (iv) a new exopinacoderm that appeared thinner than the 'neo-exopinacoderm' since fewer mesohyl cells were located on this structure. Collagen deposits were still present at and near the injury edge within the remaining areas of inflammatory cell accumulations. Within these regions, however, open spaces appeared that compressed the infiltrated mesohyl cells into thick strands (figure 19). This may have been the initiation of subdermal space reconstruction.

By 48 h, the new exopinacoderm was thin and almost normal over reforming subdermal spaces. The inflammatory infiltrate and the collagen deposits were gone. No scars remained. Newly healed areas could be identified by incomplete reconstruction of subdermal spaces, and fewer spicular projections on the new exopinacoderm which resulted in a flatter appearance of the sponge surface compared with the scalloped circus tent morphology of the normal, uninjured exopinacoderm (figure 20, plate 6).

As was found for graft rejection, archaeocytes, acidophilic and neutrophilic spherulous cells, and AMP+ cells were located in the mesohyl tracts that impinged on the injury and accumulated in the regions of choanosome damage. When differential counts of these mesohyl cells were done over time and compared to the normal composition of mesohyl cells, no substantial increase in any cell type was found (data not shown).

Mesohyl tract orientation in allografts, autografts and inflammation

Tracts or streams of cells have been noted in the mesohyl tissues of numerous sponge species responding to allografted tissues. The formation of these ephemeral structures may also be associated with growth, repair, and reconstruction, yet no quantitative analysis of the numbers or directional orientations of these structures has been reported. Therefore, on each specimen, the numbers of mesohyl tracts oriented perpendicularly or obliquely to allografted, autografted or injured areas were compared to tracts similarly oriented towards size-matched areas of unaltered exopinacoderm (figure 21). To access significance, these paired numbers were analysed by Student's paired t test, while the mean number of tracts per unit graft interface or injury edge and their respective control exopinacoderms for each time period have been included in figures 22–24. No attempt was made to quantify differences in tract size.

Allograft specimens 1 and 2 (figure 22) showed significantly more mesohyl tracts impinging on the graft interface than on the control exopinacoderm for all the time periods analysed ($p < 0.01$ or better). Specimen 3 exhibited a normal rejection response with a cellular accumulation at the graft interface. However, between 1 and 16 h, this allografted pair did not show significant numbers of mesohyl tracts oriented towards the graft interface when compared with the control exopinacoderm ($p < 0.1$). There was a slight but significant increase in the numbers of tracts oriented towards the graft interface of 24 h ($p < 0.005$) in specimen 3 when the

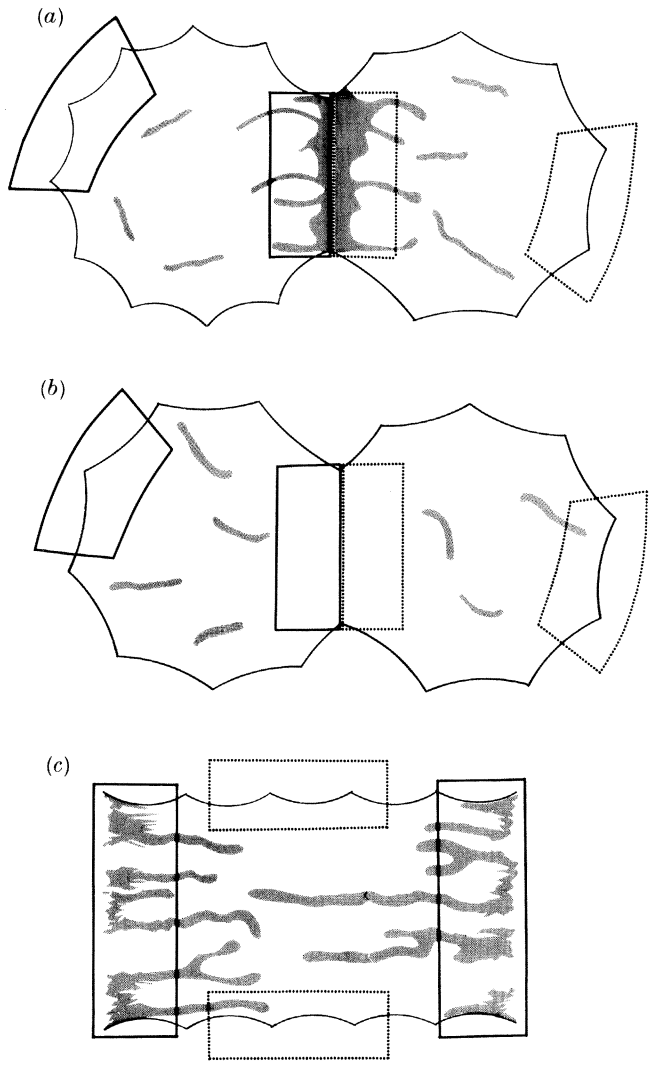


FIGURE 21. The numbers of mesohyl tracts oriented perpendicularly or obliquely towards an allograft or autograft interface or an injured edge were compared with numbers of tracts oriented towards size-matched areas of unaltered exopinacoderms on the same specimen. (*a*) Allograft, cross-section; (*b*) autograft, cross-section; (*c*) inflammation, longitudinal section.

generalized migration of mesohyl cells coalesced into a few cell tracts. Unfortunately, time periods longer than 24 h were not available. The variations in the numbers and timing of the appearance of the tracts of migrating cells in different allografted specimens may have been a cytological manifestation of the variability in rejection kinetics in this species.

Usually, the numbers and orientations of mesohyl tracts in control autografts (figure 23), unlike allografts, showed no differences between the autografted interface and ungrafted exopinacoderm ($p < 0.25$). Specimen 1, however, revealed

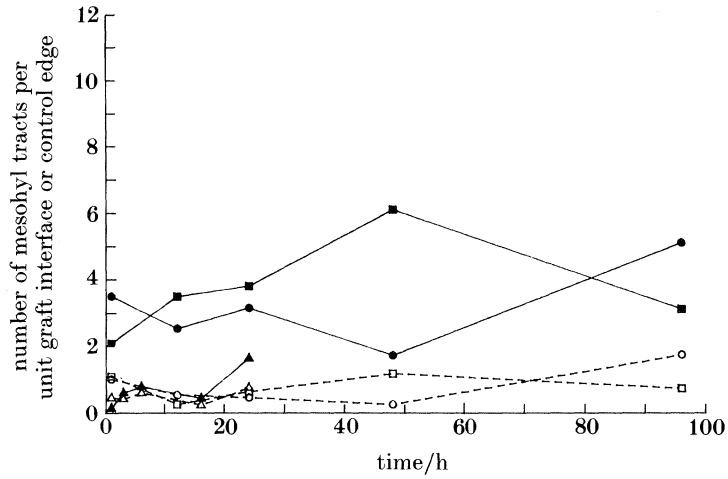


FIGURE 22. Allografts. The mean number of mesohyl tracts oriented towards the allograft interface at various time periods is compared with the mean number of mesohyl tracts oriented towards a size-matched control exopinacoderm on each specimen. Circles, specimen 1; squares, specimen 2; triangles, specimen 3; solid symbols with solid lines, experimental data; open symbols with dashed lines, control data.

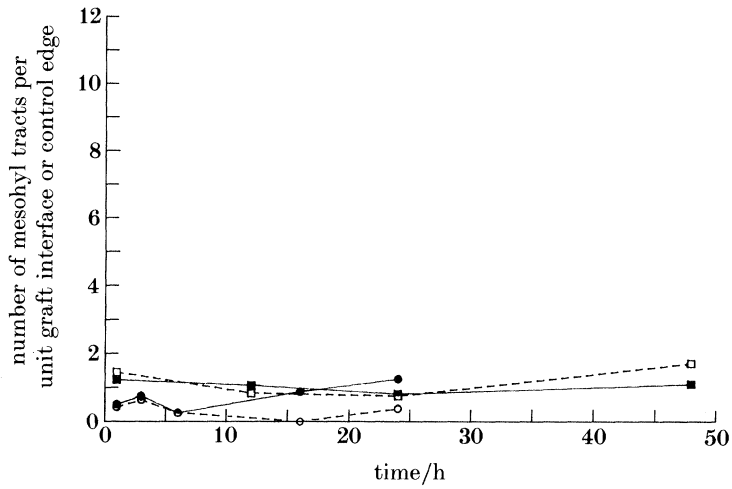


FIGURE 23. Autografts. The mean number of mesohyl tracts oriented towards the autograft interface at various time periods is compared with the mean number of mesohyl tracts oriented towards a size-matched control exopinacoderm on each specimen. Circles, specimen 1; squares, specimen 2; solid symbols with solid lines, experimental data; open symbols with dashed lines, control data.

a slight increase in tracts associated with the graft interface at 16 h ($p < 0.025$) and 24 h ($p < 0.01$). This may have been in response to the reconstruction necessary for ectosome rearrangement and choanosome fusion.

Mesohyl tracts during an inflammatory response showed significant directional orientation towards the injured areas (figure 24). Specimens 1 and 2 had peak

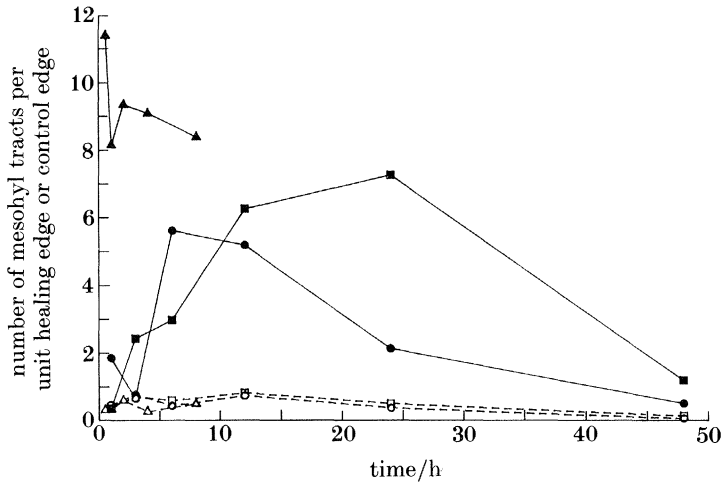


FIGURE 24. Inflammatory response to injury. The mean number of mesohyl tracts oriented towards injuries at various time periods is compared with the mean number of mesohyl tracts oriented towards size-matched control exopinacoderm on each specimen. Circles, specimen 1; squares, specimen 2; triangles, specimen 3; solid symbols and lines, experimental data; open symbols and dashed lines, control data.

numbers of tracts associated with the injured areas at 6 and 12 h, respectively, with declining numbers as healing neared completion. Specimen 3 showed high numbers of tracts associated with the injury beginning at 0.5 h. These data suggested that not all sponges responded to an injury with the same timing and magnitude. Differences may depend on the size and general health of the sponge in addition to the size and severity of the injury.

DISCUSSION

The beginnings of sponge transplantation experiments date back to the last century (Valliant 1886). This initial work, plus classical investigations of sponge cell reaggregation (Wilson 1907; Galtsoff 1925; Humphreys 1963) in addition to other transplantation studies (Moscona 1968; Müller *et al.* 1981), suggested that sponges were only capable of xenogeneic recognition and rejection. This concept was first challenged when Van de Vyver (1970) showed that the freshwater sponge *Ephydatia fluviatilis*, would not fuse with different strains of the same species, indicating allorecognition capabilities in sponges. Since then, a variety of grafting studies have indicated poriferan immune systems to be effective and diverse. Although much less is known about inflammatory responses to injury in sponges, the few species investigated responded quickly in repairing tissue damage (Jones 1957; Korotkova 1962, 1970; Harrison 1972; Boury-Esnault 1976).

Variable alloincompatibility

Two basic mechanisms of allorejection are exhibited by sponges: barrier formation and cytotoxicity (Van de Vyver 1980; Buscema & Van de Vyver 1984*b*;

Smith & Hildemann 1984). Cytotoxic rejections seem to be correlated with the presence of inducible alloimmune memory, while barrier-forming sponges do not show accelerated second set rejections (Smith & Hildemann 1984). Within these two categories, many species show varying degrees of histoincompatibility ranging from weak to strong (Hildemann *et al.* 1980; Hildemann & Linthicum 1981; Bigger *et al.* 1983; Smith & Hildemann 1984), and this has been attributed to genetic differences between the grafted sponges (Hildemann *et al.* 1979, 1980; Buscema & Van de Vyver 1984*b*; Smith & Hildemann 1984). Rejection variability appears quite pronounced in *Axinella verrucosa* where one individual can show differences in the degree of barrier formation ranging from the formation of a true barrier, to the deposition of a collagen network (which is not a barrier), to compatible allograft fusion (Buscema & Van de Vyver 1984*b*).

Allograft acceptance versus isograft fusion in sponges has been debated for some years (Curtis 1979*a*, *b*; Jokiel *et al.* 1982; Curtis *et al.* 1982; Neigel & Avise 1983; Neigel & Schmahl 1984). The basic problem arises from the unknown genetic histories including the asexual versus sexual origins of any two sponges. Higher frequencies of graft acceptances between sponges growing close together supports the postulate of isograft acceptance occurring between fragmentation (asexually) derived sponges (Jokiel *et al.* 1982; Neigel & Avise 1983; Neigel & Schmahl 1984). Previous reports of allogeneic graft acceptance have been unconvincing (Curtis 1979*a*, *b*; Kaye & Ortiz 1981; Curtis *et al.* 1982) either because putative allograft acceptances have occurred between sponges growing within a small area, or that scoring of rejections and acceptances were inconsistent (see Neigel & Avise 1985). However, Buscema & Van de Vyver (1984*b*) state that allograft acceptance in differently coloured individuals of *A. verrucosa* is cytologically different from fusion of autografts. Furthermore, Neigel & Avise (1985) demonstrate long-term graft acceptances between pairs of *Niphates erecta* that show different allozyme banding patterns.

The possibility of allograft acceptance in sponges raises questions regarding the underlying allogeneic recognition mechanisms. Allorecognition in sponges may not be an all-or-none response based on specific interactions of self-histocompatibility (H) markers and receptors for non-self on interacting allogeneic cells. Limited numbers of surface H markers or broad specificities of non-self recognition receptors might result in a continuum of cross reactivities ranging from varying magnitudes of allograft rejections to allograft acceptance.

Allocompatibility exhibited by *A. verrucosa* and *N. erecta* suggests the ability of sponges to be tolerized. Large graft doses across weak or few histocompatibility barriers tends to increase graft survival to the point, in some cases, of inducing tolerance (Hildemann *et al.* 1981). Increased rejection times have been demonstrated in a population of partly inbred sea urchins which would share many genes and H markers (Coffaro & Hindgardner 1977). Inbreeding is theoretically possible in sponge populations and natural congeneics or strains of sponges have been proposed (Kaye & Ortiz 1981), but direct information is lacking with regard to sexual and asexual reproduction, dispersal and settling patterns of sibling larvae in both *A. verrucosa* and *N. erecta* and makes arguments based on inbreeding difficult. If the sponges cited by Buscema & Van de Vyver (1984*b*) are an example

of tolerization, then the absence of any colour blending at the graft interface (cellular intermingling) by the differently coloured individual sponges is curious. It is unfortunate that these accepted allografts were not observed for longer than 15 days; tolerance in adult animals often has a finite period of effect. The basis of allograft acceptance could alternatively be a result of limited H polymorphism in some species. Congeneics at the H locus (or loci) would be a possible explanation of the prolonged graft acceptance observed between genetically dissimilar individuals of *N. erecta*.

The cell responsible for allorecognition in Callyspongia diffusa *is unknown*

It has been assumed that non-self recognition occurs in sponges upon cellular contact, but the cell type accountable for this aspect of the immune response is unknown. When a parabiosis is set up, the exopinacoderms are punctured by the opposing, protruding spicules, the pinacocytes come into contact at these points and the pinacoderms appear to fuse. Because the choanosome extends to the surface of *C. diffusa* where the spicules protrude, this initial step also brings together allogeneic mesohyl cells. Consequently, either the exopinacocytes, a mesohyl cell, or both, could be responsible for non-self recognition. This has been suggested previously by Johnston & Hildemann (1983). Because several species can reject inserted plugs of allogeneic tissues which have fewer exopinacocytes for non-self recognition (Paris 1961; Hildemann *et al.* 1980; Evans *et al.* 1980; Smith & Hildemann 1984), this indicates that the pinacocytes may not be necessary for non-self recognition and perhaps one or more of the mesohyl cells are acting in this regard. No histological information is available at early time points (less than 24 h) in graft rejection for other species. Consequently, final determination of the cell type in sponges responsible for recognizing non-self and communicating such contact will have to await further investigations.

When compared with allografts, initial contact in autografts of *C. diffusa* also involve spicular puncture of the exopinacoderms with interactions of both exopinacocytes and local mesohyl cells. Yet the ensuing autograft response consists of exopinacoderm redistribution and choanosome fusion rather than an infiltration of mesohyl cells. Substantial differences in autografts versus allografts have been reported in all other sponges investigated, both by gross morphology and by histology.

Allogeneic contact and tissue damage induce mesohyl cell migrations

A striking cytological aspect of graft rejection seen in several sponge species is the massive accumulation of cells at the graft interface (Paris 1961; Johnston & Hildemann 1983; Van de Vyver & Barbieux 1983; Buscema & Van de Vyver 1983, 1984*a*, *b*). For some species, the mesohyl cells reach the graft zone by a general movement through the choanosome (Van de Vyver & Barbieux 1983), but for others, mesohyl tracts containing cells elongated parallel to the length of the tract, are oriented towards the graft interface (Paris 1961; Johnston & Hildemann 1983; Buscema & Van de Vyver 1984*a*). The significant association of tracts with the graft zone in two of the three specimens of *C. diffusa* (figure 22) reveals that both methods of cellular migration can occur in the same species. Although no positive

control was available, the absence of mitotic figures in the graft zone supports the view that cells stream towards the area of rejection rather than accumulate as a result of proliferation (Buscema & Van de Vyver 1984*a*). Paris (1961), however, noted mesohyl tracts and mitotic archaeocytes within graft zones of *Suberites domuncula*.

Mesohyl tracts are also noted in inflammatory responses to injury in other sponges (Boury-Esnault 1976) and are found oriented towards areas of tissue damage. The inflammatory response in *C. diffusa* is mounted faster and more vigorously than that seen in graft rejections (compare figure 22 and figure 24) which suggests different or additional mechanisms for evoking inflammatory responses.

Because parabiosis does not inflict massive tissue damage during experimental transplantations in sponges, the involvement of inflammatory responses to injury in the early stages of graft rejection is probably negligible. It is the destructive final stages of rejection necrosis that might evoke such a response. However, mesohyl cell migrations towards rejection sites do not increase with the onset of cytotoxicity. In mammals, distinction between specific immunity and non-specific inflammation is based on the involvement of lymphocytes. Perhaps a similar identification of sponge immunocytes and non-specific effector cells will enable a distinction between specific and non-specific defences in sponges.

Proposed chemotactic mediators may direct cell movements

Mechanisms for directed cell movement towards sites of non-self contact are unknown in sponges. In a series of experiments on *C. diffusa*, Bigger *et al.* (1981) concluded that soluble mediators are not involved before allogeneic cell contact occurs. Yet, to explain the mechanism underlying the directed movement of mesohyl cells, their accumulation at the area of allogeneic contact and subsequent effect on tissue separation, chemotactic mediators originating at the plane of non-self contact can be postulated. Direct cell-to-cell lines of communication may be possible, but diffusable chemotactic mediators released from cells that recognize non-self cells seem more probable.

Signals that direct cells to sites of tissue damage are also unknown. In mammals, the inflammatory process is initiated by chemical mediators that are released from broken cells and damaged tissues which result in local erythema, oedema, neutrophil, monocyte and macrophage accumulations and phagocytosis of cell debris. The wound is closed by proliferating fibroblasts, with a regeneration of the damaged epithelium and capillaries (Klein 1982). Similarly, some sponge species respond quickly to injuries by migrations of cells along tracts to injured areas (Boury-Esnault 1976). The inflammatory signals to which the sponge cells respond may also originate from substances released from damaged cells, but an additional signal may be involved. Because damage can disrupt the water-tight aquiferous canal system and alter the pumping efficiency (Ankel 1965), changes in the water pressure in this system may also serve to pinpoint the site of damage.

Cell types involved in inflammation

A relatively normal makeup of mesohyl cells appears to migrate towards sites of tissue damage in *C. diffusa* which include mostly archaeocytes with acidophilic

and neutrophilic spherulous cells and AMP+ cells (see Smith 1985). Archaeocytes have been noted as the major responder–effector cell in wound healing for *Polymastia mamillaris* (Boury-Esnault 1976). This cell type accumulates at the site of injury, phagocytoses damaged cells and replaces lost exopinacoderm as suggested by the appearance of pinacocytes with archaeocyte-like characteristics (Boury-Esnault 1976). The archaeocytes of *C. diffusa* also phagocytose cell debris, but their potential for exopinacocyte regeneration is inconclusive since their nuclear morphology and cytoplasmic staining characteristics are already similar to those of the pinacocytes (Smith 1985).

Not all sponges mount inflammatory responses with archaeocyte infiltration and archaeocyte replacement of lost cell types. Other species respond to injuries with increases in local mitotic rates which regenerate lost cells from the same cell type, and close the wound by ingrowth (Jones 1957; Korotkova 1962, 1970). In general, sponges appear to repair tissue damage either by an infiltration of archaeocytes and other mesohyl cells, or by local increases in mitoses that result in ingrowth.

TABLE 1. CELL TYPE IN REJECTION ZONE CORRELATES WITH MODE OF REJECTION IN SPONGES

species	cellular infiltrate	mesohyl tracts	barrier construction	cyto-toxicity	reference
Hymeniacidon perleve	archaeocytes	?	no	yes	Evans *et al.* 1980
Callyspongia diffusa	mesohyl cells† (91% archaeocytes)	yes	no	yes	this paper, Johnston & Hildemann 1983
Axinella polypoides	archaeocytes	yes	no‡	yes	Buscema & Van de Vyver 1984*a*
Suberites domuncula	amoebocytes§ (polyblasts)	yes	?	?‖	Paris 1961
Axinella damicornis	collencytes spherulous cells	no	yes	no	Buscema & Van de Vyver 1984*b*
Axinella verrucosa					Buscema & Van de Vyver 1983, 1984*b*
chronic	collencytes	no	yes	no	
non-fusion	collencytes	no	yes‡	no	
fusion¶	collencytes archaeocytes	no	no	no	
Polymastia mamillaris	collencytes archaeocytes	no	no††	no	Van de Vyver & Barbieux 1983
Polymastia robusta	collencytes archaeocytes grey cells	no	no††	no	Van de Vyver & Barbieux 1983

† Collencytes are not present in this species.
‡ A collagen network is secreted that is not a barrier.
§ These are other names for archaeocytes.
‖ It has been predicted that this species would give a cytotoxic response because of the archaeocyte infiltrate (Smith & Hildemann 1984).
¶ The ectosomes are reorganized and the choanosomes fuse compatibly.
†† Dense collagen fibres are deposited between the allogeneic cells that line up on either side of the graft interface.

Different cell types are involved in different types of graft rejection

Two very different types of rejections have been noted in sponges: barrier formation and cytotoxicity (Van de Vyver 1980; Buscema & Van de Vyver 1984*b*; Smith & Hildemann 1984). Underlying reasons for this may be based upon the types of mesohyl cells normally present within a sponge species. There appears to be a correlation between the presence, absence or involvement of collencytes in rejection zones and the mode of rejection that is exhibited (table 1). When archaeocytes respond and migrate to an area of allogeneic contact, either because the collencytes are unresponsive or absent (*C. diffusa* does not have collencytes (Smith 1985)), the result is cytotoxicity (Evans *et al.* 1980; Johnston & Hildemann 1983; Buscema & Van de Vyver 1984*a*). When collencytes respond, with or without a simultaneous archaeocyte migration, collagen secretion ensues at the interface with the formation of a barrier or a dense deposit (Van de Vyver & Barbieux 1983; Buscema & Van de Vyver 1983, 1984*b*). This predicts that *Aplysina* (*Verongia*) *longissima* (Kaye & Ortiz 1981; Neigel & Avise 1983), a species that forms barriers in response to non-self contact, would have collencyte-mediated rejections, and that *Toxadocia violacea* (Bigger *et al.* 1983) and *Xestospongia exigua* (Hildemann & Linthicum 1981), both of which show cytotoxic reactions to non-self, would have archaeocyte-mediated responses. Furthermore, the positive correlation between cytotoxic allograft rejection and demonstrable alloimmune memory (Smith & Hildemann 1984), predicts that archaeocytes or an archaeocyte subpopulation are responsible for short-term alloimmune memory.

Conclusion

The effectiveness of sponges to reject non-self tissues and to repair tissue damage are two characteristics that reflect the survivability of the members of this phylum. In habitats where allogeneic contact can occur and where xenogeneic contact (with prokaryotes, protozoans and metazoans) occurs continually, sedentary sponges must have protective mechanisms to guard against invasion of self tissues. Injury-induced exposure of the internal region of a sponge, the mesohyl, to the external environment is a serious situation for a filter-feeding organism. Bacteria are one of the major components of the filtrate that sponges use as a nutrient source (Reiswig 1975), and many sponges even maintain controlled symbiotic relationships with certain prokaryotes (Vacelet & Donaday 1977; Castro 1979). A disruption of the compartmentalization of the sponge body could potentially lead to an invasion of the mesohyl by an opportunistic microbe present in the aquiferous system that would otherwise be kept in check by the sponge organization and defence system. An infection coupled with a decrease in feeding efficiency from a disruption of water flow could be most disastrous for a sponge. The abilities of *C. diffusa* and other species to control infection (xenogeneic contact) and maintain self integrity against natural tissue transplantation in the marine benthic environment has been inferred from the impressive inflammatory and graft rejection responses that sponges mount towards artificially induced injuries and allografts.

Paul Jokiel and Russel Ito helped collect sponges around Coconut Island. Expert technical assistance was offered by Douglas Doyle, Joyce Schuman with Dr Charles Bigger helping in many aspects of this study. The manuscript was critically read by Dr E. Cooper, Dr J. Neigel, Dr C. Bigger, Dr J. Bramhall, Dr L. Muscatine and Dr E. Stein. This work was supported by National Institutes of Health grants AI 15075 and AI 19470.

References

Ankel, W. E. 1983 Der Susswassweschwämm *Ephydatia fluviatilis* (Einleitende Worte zur Vorführung des Films). *Zool. Anz.* **28** (suppl.), 426–444.

Bigger, C. H., Hildemann, W. H., Jokiel, P. L. & Johnston, I. S. 1981 Afferent sensitization and efferent cytotoxicity in allogeneic tissue responses of the marine sponge *Callyspongia diffusa*. *Transplantation* **31**, 461–464.

Bigger, C. H., Jokiel, P. L., Hildemann, W. H. & Johnston, I. S. 1982 Characterization of alloimmune memory in a sponge. *J. Immun.* **129**, 1570–1572.

Bigger, C. H., Jokiel, P. L. & Hildemann, W. H. 1983 Cytotoxic transplantation immunity in the sponge *Toxadocia violacea*. *Transplantation* **35**, 239–243.

Buscema, M. & Van de Vyver, G. 1983 Variability of allograft rejection processes in *Axinella verrucosa*. *Devl. comp. Immunol.* **7**, 613–616.

Buscema, M. & Van de Vyver, G. 1984*a* Cellular aspects of alloimmune reactions in sponges of the genus *Axinella*. I. *Axinella polypoides*. *J. exp. Zool.* **229**, 7–17.

Buscema, M. & Van de Vyver, G. 1984*b* Cellular aspects of alloimmune reactions in sponges of the genus *Axinella*. II. *Axinella verrucosa* and *Axinella damicornis*. *J. exp. Zool.* **229**, 19–32.

Boury-Esnault, N. 1976 Morphogenèse expérimentale des papilles inhalantes de l'éponge *Polymastia mamillaris* (Muller). *Arch. Zool. exp. Gén.* **117**, 181–196.

Castro, P. 1979 Studies on the symbiosis between a filamentous microorganism and *Hymenamphiastra cyanocrypta*, a sponge from California. In *Biologie des Spongiares* (ed. C. Levi & N. Boury-Esnault), Colloq. int. du C.N.R.S., no. 291, pp. 365–371. Paris: Editions du C.N.R.S.

Coffaro, K. A. & Hinegardner, R. T. 1977 Immune response in the sea urchin *Lytechinus pictus*. *Science, Wash.* **197**, 1389–1390.

Curtis, A. S. G. 1979*a* Individuality and graft rejection in sponges or a cellular basis for individuality in sponges. In *Biology and systematics of colonial organisms* (ed. G. Larwood & B. R. Rosen), Systematics Association Special vol. no. 11, pp. 39–48. New York: Academic Press.

Curtis, A. S. G. 1979*b* Recognition by sponge cells. In *Biologie des Spongiares* (ed. C. Levi & N. Boury-Esnault), Colloques int. du C.N.R.S., vol. no. 291, pp. 205–209. Paris: Editions du C.N.R.S.

Curtis, A. S. G., Kerr, J. & Knowlton, N. 1982 Graft rejection in sponges. *Transplantation* **33**, 127–133.

Evans, C. W., Kerr, J. & Curtis, A. S. G. 1980 Graft rejection and immune memory in marine sponges. In *Phylogeny of immunological memory* (ed. M. J. Manning), pp. 27–34. Amsterdam: Elsevier–North-Holland Biomedical Press.

Galtsoff, P. S. 1925 Regeneration after dissociation (an experimental study of sponges). II. Histogenesis of *Microciona prolifera* Verr. *J. exp. Zool.* **42**, 223–251.

Harrison, F. W. 1972 The nature and role of the basal pinacoderm of *Corvomeyenia carolinensis* Harrison (Porifera: Spongillidae). A histochemical and developmental study. *Hydrobiologia* **34**, 495–508.

Hildemann, W. H., Johnston, I. S. & Jokiel, P. L. 1979 Immunocompetence in the lowest metazoan phylum: transplantation immunity in sponges. *Science, Wash.* **204**, 420–422.

Hildemann, W. H., Bigger, C. H., Johnston, I. S. & Jokiel, P. L. 1980 Characterization of transplantation immunity in the sponge *Callyspongia diffusa*. *Transplantation* **30**, 362–367.

Hildemann, W. H. & Linthicum, D. S. 1981 Transplantation immunity in the Palaun sponge, *Xestospongia exigua*. *Transplantation* **32**, 77–80.

Hildemann, W. H., Clark, E. A. & Raison, R. L. 1981 *Comprehensive immunogenetics*. New York: Elsevier–North-Holland Inc.

Humphreys, T. 1963 Chemical dissociation and *in vitro* reconstruction of sponge cell adhesions. I. Isolation and functional demonstration of the components involved. *Devl. Biol.* **8**, 27–47.

Johnston, I. S., Jokiel, P. L., Bigger, C. H. & Hildemann, W. H. 1981 The influence of temperature on the kinetics of allograft reactions in a tropical sponge and a reef coral. *Biol. Bull. mar. biol. Lab., Woods Hole* **160**, 280–291.

Johnston, I. S. & Hildemann, W. H. 1982 Cellular defense systems of the porifera. In *The reticuloendothelial system*, vol. 3 (ed. N. Cohen & M. M. Sigel), pp. 37–57. New York: Plenum.

Johnston, I. S. & Hildemann, W. H. 1983 Morphological correlates of intraspecific grafting reactions in the marine demosponge *Callyspongia diffusa*. *Mar. Biol.* **74**, 25–33.

Jokiel, P. L., Hildemann, W. H. & Bigger, C. H. 1982 Frequency of intercolony graft acceptance or rejection as a measure of population structure in the sponge *Callyspongia diffusa*. *Mar. Biol.* **71**, 135–139.

Jones, W. C. 1957 The contractility and healing behavior of pieces of *Leucosolenia complicata*. *Q. Jl. microsc. Sci.* **98**, 203–217.

Kaye, H. & Ortiz, T. 1981 Strain specificity in a tropical marine sponge. *Mar. Biol.* **63**, 165–173.

Klein, J. 1982 *Immunology: the science of self–non-self discrimination*. New York: John Wiley.

Korotkova, G. P. 1962 Behavior of the cellular elements in the calcareous sponge *Leucosolenia complicata* Mont. during regeneration. *Acta biol. hung.* **13**, 1–30.

Korotkova, G. P. 1970 Regeneration and somatic embryogenesis in sponges. In *The biology of the Porifera* (ed. W. G. Fry). *Symp. zool. Soc. Lond.* **25**, 423–436. London: Academic Press.

Moscona, A. A. 1968 Cell aggregation: properties of specific cell ligands and their role in the formation of multicellular systems. *Devl. Biol.* **18**, 250–277.

Müller, W. E. G., Bernd, A., Zahn, R. K., Kurlec, B., Daves, K., Müller, I. & Uhlenbruck, G. 1981 Xenograft rejection in marine sponges. Isolation and purification of an inhibitory aggregation factor from *Geodia cydonium*. *Eur. J. Biochem.* **116**, 573–579.

Neigel, J. E. & Avise, J. C. 1983 Histocompatibility bioassays of population structure in marine sponges. *J. Hered.* **74**, 134–140.

Neigel, J. E. & Avise, J. C. 1985 The precision of histocompatibility response in clonal recognition in tropical marine sponges. *Evolution* **39**, 724–732.

Neigel, J. E. & Schmahl, G. E. 1984 Phenotypic variation within histocompatibility-defined clones of marine sponges. *Science, Wash.* **224**, 413–415.

Paris, J. 1961 Greffes et serologie chez les éponges siliceuses. *Vie et Milieu*, suppl. no. 11.

Randal, J. E. & Hartman, W. D. 1968 Sponge-feeding fishes of the West Indies. *Mar. Biol.* **1**, 216–225.

Reiswig, H. M. 1975 Bacteria as food for temperate-water marine sponges. *Can. J. Zool.* **53**, 582–589.

Resh, J. H. 1976 Life cycles of invertebrate predators of fresh water sponge. In *Aspects of sponge biology* (ed. F. W. Harrison & R. R. Cowden), pp. 229–314. New York: Academic Press.

Simpson, T. L. 1984 *The cell biology of sponges*. New York: Springer-Verlag.

Smith, L. C. 1985 Cellular defense systems in sponges. Ph.D. dissertation, University of California, Los Angeles.

Smith, L. C. & Hildemann, W. H. 1984 Alloimmune memory is absent in *Hymeniacidon sinapium*, a marine sponge. *J. Immun.* **133**, 2351–2355.

Smith, L. C. & Hildemann, W. H. 1986 Allogeneic cell interactions during graft rejection in *Callyspongia diffusa* (Porifera; Demospongia); a study with monoclonal antibodies. *Proc. R. Soc. Lond.* B **226**, 465–477.

Stone, A. R. 1970 Growth and reproduction of *Hymeniadcidon perleve* (Montague) (Porifera) in Langston Harbour, Hampshire. *J. Zool., Lond.* **101**, 443–459.

Valliant, L. 1886 Note sur la vitalité d'une éponge de la famille des Corticatae la *Tethya lyncurium*, Lamarck. *C. r. hebd. Séanc. Acad. Sci., Paris* **68**, 86–88.

Vacelet, J. & Donaday, C. 1977 Electron microscope study of the association between some sponges and bacteria. *J. exp. mar. Biol. Ecol.* **30**, 301–314.

Van de Vyver, G. 1970 La non confluence intraspecifique chez les spongiaires et la notion d'individu. *Ann. Embryol. Morphol.* **3**, 251–262.

Van de Vyver, G. 1980 Second set allograft rejection in two sponge species and the problem of an alloimmune memory. In *Phylogeny of immunological memory* (ed. M. J. Manning), pp. 15–26. Amsterdam: Elsevier–North-Holland Biomedical Press.

Van de Vyver, G. & Barbieux, B. 1983 Cellular aspects of allograft rejection in marine sponges of the genus *Polymastia*. *J. exp. Zool.* **227**, 1–7.

Wilson, H. V. 1907 On some phenomena of coalescence and regeneration in sponges. *J. exp. Zool.* **5**, 245–258.