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Comparison of Discharge Mechanisms of Cnidarian Cnidae and Myxozoan Polar Capsules

Quinn Cannon and Eric Wagner
Fisheries Experiment Station, Utah Division of Wildlife Resources, 1465 West 200 North, Logan, Utah 84321, USA

ABSTRACT: Discharge stimuli and cell structure of polar capsules and cnidae are reviewed for both Cnidaria and Myxozoa. The discharge process in Cnidaria (especially Anthozoa) can be summarized as follows: 1) cnidae can discharge either independently or under the influence of adjacent cells. There are at least three types of cell complexes: one responding to mechanical stimuli, one to mechanical stimuli only after chemosensitization, and another responding to vibrational frequencies. 2) Supporting cells for the complex requiring chemosensitization bear chemoreceptors to mucins, N-acetylated sugars, and certain proteins. Binding of these ligands modifies the length of sensory cilia, sensitizing the cnidocyte to mechanical stimuli. 3) Mechanical stimuli from prey triggers the sensitized cnidocyte and cnidae discharge; spirocyst tubes stick to prey and penetrating nematocysts puncture prey. 4) Body fluids from prey provide additional ligands, further influencing cnidocytes. 5) Venom from nematocyst increases struggling of prey, mechanically triggering more nematocysts. Chemical signaling between cells is reviewed. Discharge in Myxozoa is less studied, but can occur after treatment with strong bases, urea, concentrated hydrogen peroxide, potassium salts, and in some species, mucus. Similarities between Cnidaria and Myxozoa include radial symmetry, development of specialized structures containing inverted, helically coiled filaments that are sticky and capped with a "stopper," and the filament discharge response of these structures to pressure, potassium ion, and extreme pH. Myxozoans differ from cnidarians in the lack of a nerve net, less cellular organization, different life cycle stages, and in the response to chemosensitizers, neurochemicals, and external Ca^{2+} removal.

KEY WORDS: Myxozoa, nematocyst, polar filament, cnidae, eversion, polar capsule, Cnidaria.

I. INTRODUCTION

Cnidaria (= Coelenterata) is the animal phylum characterized by radial symmetry about an oral-aboral axis, lack of a head or segmentation, and a body consisting of two cell layers (epidermis and gastrodermis) with mesoglea between them (Storer *et al.*, 1972). Cnidaria is divided into three classes: Anthozoa (*e.g.*, sea anemones,

corals), Hydrozoa (*e.g.*, hydra, Portuguese man-of-war), and Scyphozoa (jellyfishes). All cnidarians are aquatic and most are marine (Barnes *et al.*, 1993). The stinging cells of Cnidarians, used for prey capture, defense, substrate attachment, and other functions, are called cnidocytes (McKay and Anderson, 1988). Cnidocytes synthesize cnidae, which are intracellular secretory products diagnostic of all Cnidarians (Watson and Wood, 1988). Nematocysts, spirocysts, and ptychocysts are the three categories of cnida (Mariscal, 1984; Watson and Wood, 1988).

Myxozoa is the animal phylum characterized by spores made of several cells forming one to seven shell valves, one to two amoeboid infective sporoplasms, and two to seven polar capsules (Lom and Dyková, 1992). The polar capsules contain an extrudible polar filament that serves to anchor the parasite to the host tissue. The polar capsule is similar to the nematocyst in this regard. This article summarizes some of the similarities and differences between the two structures in form, as well as triggers for firing of the extrudible filaments. Given some of the homology between the structures, information discovered by researchers working on Cnidaria may provide insight into possible mechanisms for firing of polar filaments of Myxozoans. This information could be useful in the control effort against serious fish pathogens such as *Myxobolus cerebralis*, the causative agent of whirling disease.

II. CNIDAE STRUCTURE

Cnidae are secretory products of cnidocytes, arising from the Golgi apparatus and endoplasmic reticulum (Slautterback and Fawcett, 1959) and consists of a capsule containing a highly folded, eversible tubule (Watson and Wood, 1988; Figure 1). Cnidae are not regenerated by the cnidocytes after exocytosis; instead new cnidocytes are formed (Tardent, 1995). The capsule is made of a relatively thick elastic substance and has a "mouth" closed by an operculum, or "lid" (Kepner *et al.*, 1951). The capsules of undischarged nematocysts are generally either rod-like or spherical, and their sizes range from about 3 to over 100 μm in length (Mariscal, 1974). The capsule wall construction varies with development and among species. For example, in *Physalia physalis*, *Metridium senile*, and *Aiptasia pallida*, mature nematocysts contained many disulfide linkages (Blanquet and Lenhoff, 1966; Brand *et al.*, 1993; Goldberg and Taylor, 1997), whereas smaller capsules, spirocysts, and nematocysts of the black coral *Cirrhipathes luetkeni* did not (Goldberg and Taylor, 1997). Inside the capsule is a fluid that contains a high concentration of solutes and a high calcium concentration (Lubbock *et al.*, 1981). Based on the freezing point depression of capsules, Picken and Skaer (1966) suggested that osmotic pressure within may be as high as 140 bar. Using refractive index measurements, Lubbock et al. (1981) estimated the dry mass of solutes to be about 25–30%. The solutes within the capsules are a complex mix of large polypeptides, mediators of inflammation (*e.g.*, histamine, seretonin, prostaglandins), glutamic acid, glutamine, and enzymes (Burnett and Calton, 1977; Phelan and Blanquet, 1985). The venom in only two nematocysts was sufficient to kill a mosquito larvae within seconds (Tardent, 1995).

The tubule is the cylindrical, hollow portion of the cnida that is attached to the apex of the capsule and everts during discharge (Watson and Wood, 1988). The tubule is coiled and pleated, with three flaps that form during inversion as the cnida develops (Skaer, 1973; Watson and Mariscal, 1985). Upon discharge, the opercular

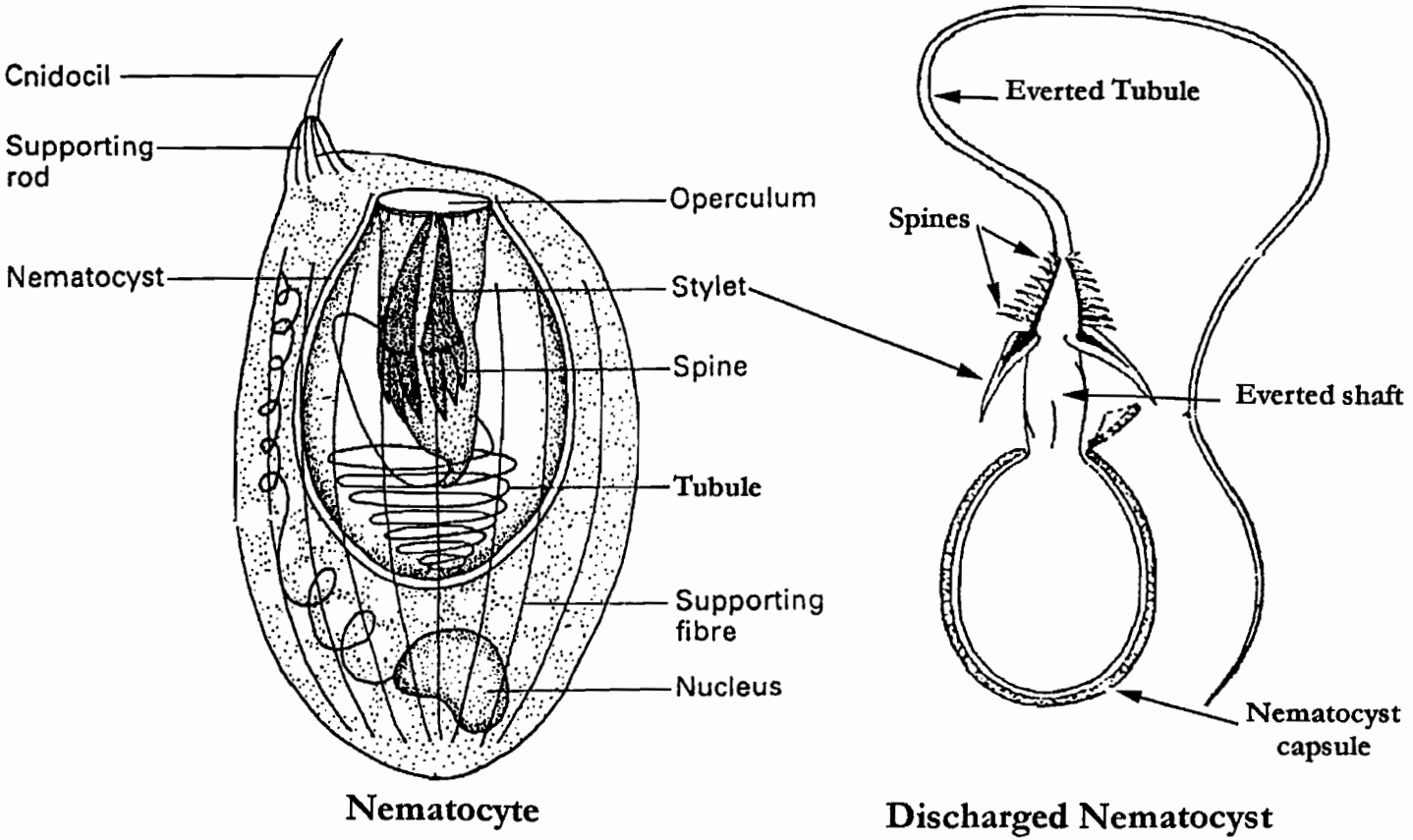

FIGURE 1. Schematic illustration of a stenotele showing general morphological characteristics common to many cnidocytes (after Barnes *et al.*, 1993; Storer *et al.*, 1972). Reprinted, with permission, from Blackwell Science Ltd.

flaps open and the tubule is released, everting out of its inverted position (Figure 1). Based largely on nematocyst morphology, *e.g.*, the presence or absence of spines, ridges, or denticles on external filaments, over 25 categories of nematocysts have been recognized (Calder, 1982; Figure 2). However, individuals may have multiple types of nematocysts that function in slightly different ways and respond to stimuli differently.

Spirocysts, which are found only in zoantharian anthozoans and outnumber nematocysts by a factor of 2 to 3, differ chemically and morphologically from nematocysts (Thorington and Hessinger, 1990). Mariscal (1974) suggested that spirocysts serve to hold prey, while the penetrating nematocysts discharge and inject a drop of venom. Spirocysts are characterized by a thin, single-walled, acidophilic capsule and contain a long, spirally coiled, unarmed tubule of uniform diameter (Mariscal, 1974; Mariscal *et al.*, 1976) (Figure 3). Spirocyst tubules bear hollow rods that dissociate upon discharge, forming an adhesive web of microfibers that adhere to prey after discharging (Mariscal *et al.*, 1977b).

Ptychocysts are found only in ceriantharian anemones and are characterized by elaborately pleated tubules that lack spines (Mariscal *et al.*, 1977a). The undischarged filament differs from other nematocysts in that the tubule is not helically folded within the capsule and the tubule tip is closed (Mariscal *et al.*, 1977a). The filaments are also adhesive and assist in forming the tube in which ceriantharians are encased (Barnes *et al.*, 1993).

Anthozoa, which includes all of the sea anemones, has six different types of nematocysts: basitrichous isorhizas, atrichous isorhizas (= stereoline glutinant), holotrichous isorhizas (= streptoline glutinant), microbasic mastigophores, microbasic amastigophores, and macrobasic amastigophores (Mariscal, 1974) (Figure 2). Microbasic amastigophores and macrobasic amastigophores are unique to Anthozoa (Mariscal, 1974). Holotrichous isorhizas are affixing nematocysts with long tubules bearing minute thorns (Kepner *et al.*, 1951). An atrichous isorhiza is slightly smaller than the holotrichous isorhiza and their external filaments have a much larger diameter (Kepner *et al.*, 1951). They produce sticky secretions that assist in prey capture (Kepner *et al.*, 1951).

Hydrozoa has 23 types of nematocysts, 17 of which are unique. Nematocysts which are unique to class Hydrozoa include: stenoteles (also known as penetrants), desmonemes (= volvent), anacrophores, acrophores, spiroteles, aspiroteles, merotrichous isorhizas, apotrichous isorhizas, atrichous anisorhizas, macrobasic mastigophores, semiophoric microbasic euryteles, telotrichous macrobasic euryteles, merotrichous macrobasic euryteles, holotrichous macrobasic euryteles, and birhopaloides (Mariscal, 1974) (Figure 2). Unlike stenoteles, the spheroidal desmonemes (volvents) do not vary much in size and lack stylets and lamellae. These occur in great numbers upon the tentacles of polyps. The capsule itself is 9 μm in diameter and contains a short, thick tubule in a single loop. This tubule is used to wrap tightly around prey upon discharge.

Among the Scyphozoans, only three types of nematocysts have been identified (Calder, 1982). These three nematocysts are not unique to Scyphozoa and include holotrichous isorhizas, heterotrichous anisorhizas and heterotrichous microbasic euryteles (Calder, 1982). Holotrichous isorhizas and heterotrichous microbasic euryteles are present in the planula, scyphistoma, ephyra, and medusa stages of scyphozoans (Calder, 1982).

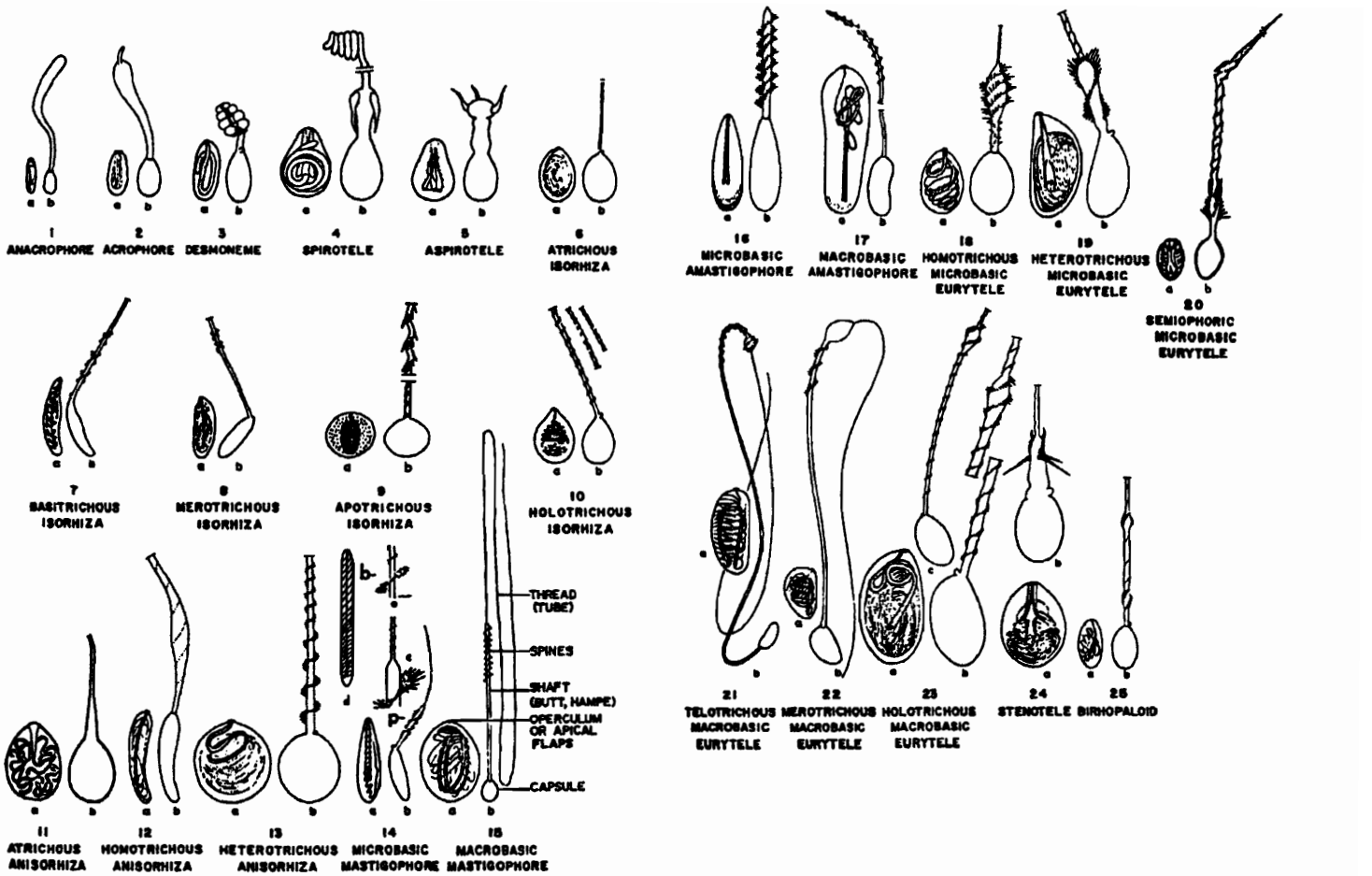

FIGURE 2. Schematic illustrations of the various types of nematocysts described to date (after Weill, 1934, 1964; Carlgren, 1940; Cutress, 1955; Hand, 1961; Mackie and Mackie, 1963; Werner, 1965; Deroux, 1966; Lacassagne, 1968a, 1968b; Mariscal, 1972, 1974). Reprinted, with permission, from Academic Press.

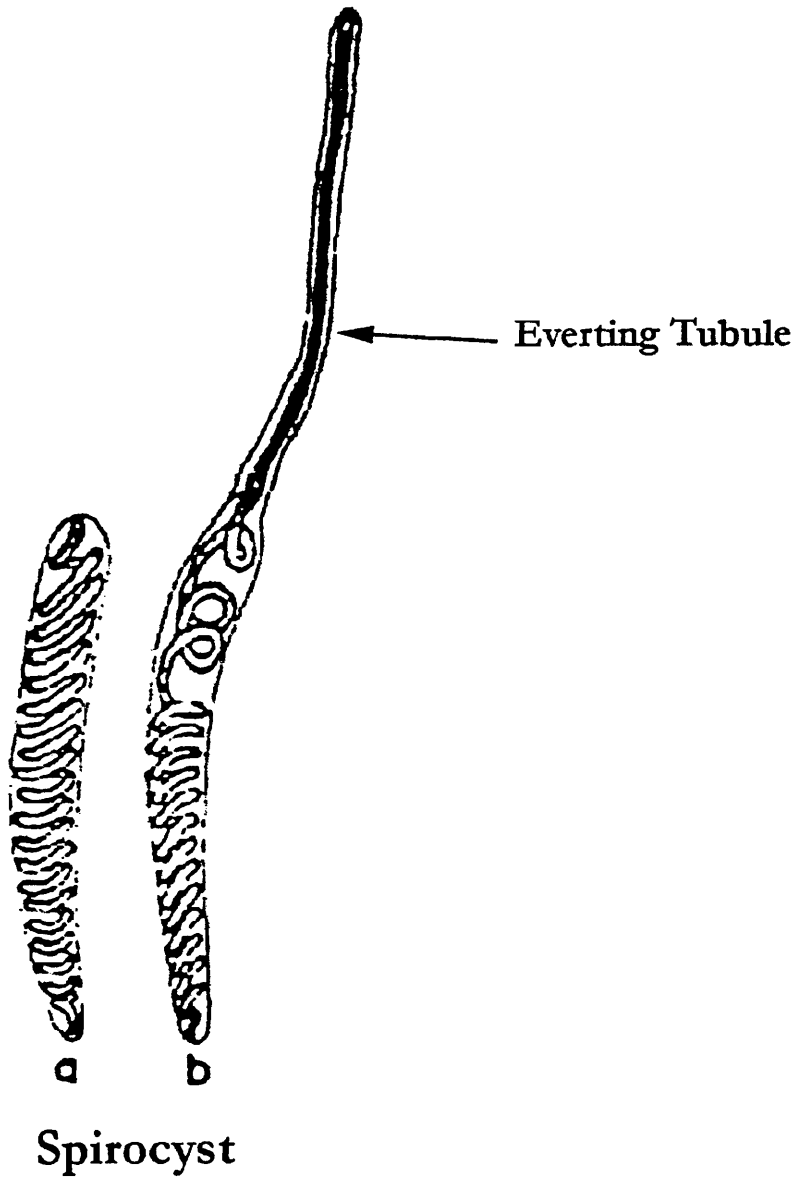

FIGURE 3. Schematic illustration of a spirocyst (a) before discharge of tubule and (b) after discharge of tubule (after Mariscal, 1974). Reprinted, with permission, from Academic Press.

III. CNIDAE DISCHARGE

A. EARLY THEORIES

Cnidae and their discharge have puzzled observers ever since this unique cellular structure was discovered. Cnidae discharge is considered to be one of the fastest

exocytotic actions (Holstein and Tardent, 1984); the entire discharge process can happen in only 3 milliseconds. It has been difficult to determine where, or how the nematocyst obtains the energy required to discharge at such a rapid speed. There have been a number of hypotheses formed about the source of energy used by discharging nematocysts, but only a combination of a few are generally accepted today. The intrinsic forces hypothesis proposes that energy is built-up during cnidogenesis and stored within the cyst walls (Dujardin, 1845; Jones, 1947; Carré, 1980). The alternate "osmotic theory" credits discharge to osmotically generated intracapsular hydrostatic pressure which increases immediately before and/or during the event of discharge by swelling of the cyst's matrix (Iwanzoff, 1896; Picken, 1953; Slautterback, 1963; Gupta and Hall, 1984). It has also been found that the tubule itself enlarges and elongates due to osmotic pressure just prior to discharge (Kepner *et al.*, 1951). The nematocyst capsule increased about 10% in size at this time (Tardent, 1988), supporting the latter theory. Tardent (1988) suggested that both osmotically generated intracapsular hydrostatic pressure and intrinsic forces produce the energy required for discharge. However, evidence suggests that conformational changes or dissociation of protein molecules may also play a role (Salleo *et al.*, 1986). Slautterback (1963) and Watson and Mariscal (1985) observed that microtubules form the scaffolding around the developing nematocyst and later form a tight skein around the developed nematocyst. An extracellular network of fibers that appeared to contact the ciliary cone receptor apparatus for the nematocyst was noted above the capsule tip. Contraction of these fibers could aid in the process of discharge. Picken (1953) and Mariscal (1974) suggested that swelling and expansion of the inverted tubule could create enough intracapsular pressure to induce discharge.

B. DISCHARGE AGENTS

Many experiments have explored chemical and mechanical stimuli that effectively discharge cnidae, though the effective stimuli depends on whether the cnidae are *in-situ* (Table 1) or isolated (Table 2). Stimuli such as human hair, human skin, some food objects, and longer chained fatty acids (especially stearic acid, no concentration given) were very effective for discharging *in-situ* nematocysts. However, Thorington and Hessinger (1988) found that short-chained fatty acids like acetic and butyric acids at low concentrations (<0.5%) had no effect on discharge. Thorington and Hessinger (1988) also found that lipids required a high chloride ion (no concentration given) content in surrounding medium to have any effect on nematocysts. A 1% solution of Na-laurylsulfate immediately discharged isolated nematocysts, whereas lower concentrations took longer or had no effect (Yanagita, 1960b). Other surface actants that were anion-active were also effective, as well as the dyes malachite green, methyl violet, methyl green, and Hoffmann violet in concentrations of about 1% (Parker, 1916; Yanagita, 1960b). Crustacean body fluid increased discharge of spirocysts of *Diadumene luciae* after mechanical stimulation (Williams, 1968). Other discharge stimuli included solutions of chloretone mixed in sea water, mineral acid, extract from the skin of the common killfish *Fundulus heteroclitus*, juice from the clam *Venus mercenaria*, 1 g sugar/mL seawater, and ether (Parker and Van Alstyne, 1932). Parker and Van Alstyne also noted that the skins of some fish lack substances

TABLE 1
Compilation of Research on Discharge of In Situ Cnidae

Class Genus	Chemical/Force	Concentration	Discharge	Authors	Other Notes
Anthozoa *Aiptasia*	KCl in Ca^{2+}-free ASW	553 mM	+	Santoro and Salleo, 1991	partial discharge
	KCl + Ca^{2+} in ASW	553 mM	−		
	NaSCN in Ca^{2+}-free ASW	553 mM	+		partial discharge
	NaSCN + Ca^{2+} in ASW	553 mM	+		total discharge
	NaI in Ca^{2+}-free ASW	553 mM	+		partial discharge
	NaI + Ca^{2+} in ASW	553 mM	+		total discharge
Anemonia	seawater & HCl solution	pH 2 or below	+	Pantin, 1942	
	KCl (isotonic) & seawater	≥5% of 0.6 M/KCl	−		cnida in tentacles
	KCl (isotonic) & seawater	40% of 0.6 M/KCl	+		large number discharged, but not majority
	asparagine	saturated	−		
	cystine	saturated	−		
	tyrosine	saturated	−		
	arginine	10%	−		
	glucose	10%	−		
	saccharose	10%	−		
	glyceral	10%	−		
	aspartic acid	saturated	−		
	histidine	saturated	−		
	glutamic acid	10%	−		
	lactose	10%	−		
	creatine	saturated	−		
	leucine	saturated	−		
	glycine	10%	−		
	laevulose	10%	−		
	cystein	saturated	−		
	tryptophane	saturated	−		

Anemonia	proline	10%	−	Pantin, 1942	
	maltose	10%	−		
	acetic acid	0.1%, pH 4–4.6	+		
	butyric acid	0.1%, pH 4–4.6	+		
	caproic acid	0.1%, ph 4–4.6	+		
	caprylic acid	0.1%, ph 4–4.6	+		
	ethyl alcohol	3–10%	+		
	isopropyl alcohol	3–10%	+		
	butyl (C_4) alcohol	3%	+		
	amyl (C_5) alcohol	2%	+		
	quinine	0.1%	+		spontaneous discharge slow & incomplete
	saponin	0.1%	+		every cnidoblast in tentacle discharged
	Na taurocholate	0.1%	+		every cnidoblast in tentacle discharged
	Na glycocholate	0.1%	+		every cnidoblast in tentacle discharged
	bile salt	1%	+		
	human saliva in sea water	0.5% dry weight of human saliva	+		no solid particles in solution
	Na taurocholate	1 drop 1%	+		
	egg albumen in sea water	10% by dry weight	+		
	Witte's peptone in sea water	10% by dry weight	+		
Calliactis parasitica	KCl (isotonic) & seawater	≥5% of 0.6 M/KCl	+	Pantin, 1942	cnida in acontium
Diadumene luciae	sea water diluted ≥20 times with distilled H_2O		+	Yanagita, 1959b	tested microbasic p-mastigophores; whole cnida extruded
	saponin	0.01%	+	Yanagita, 1960b	mixed in sea water
	digitalin	0.01%	+		

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TABLE 1
Compilation of Research on Discharge of In Situ Cnidae *(Continued)*

Class Genus	Chemical/Force	Concentration	Discharge	Authors	Other Notes
	Na-taurocholate	0.15%	+		
	Na-glycocholate	0.1%	+		
	Na-oleate	0.1%	+		
	Na-ricinoleate	0.01%	+		
	Na-laurylsulfate	0.05%	+		
	Na-oleoylmethyltaurate	0.01%	+		
	Na-isopropyl-naphthalenesulfonate	0.3%	+		
	Cetyltrimethyl-ammonium bromide	0.1%	+		
	Cetylmethyl-pyridinium bromide	0.1%	+		
	Octadecyl ether of hepta-ethylene glycol	5.0%	+		
	anilin dyes	1%	+		*e.g.*, malachite green & methyl violet
	Amphipod or Isopod body fluid		+	Williams, 1968	Spirocyst discharge threshold lowered
Metridium	HCl	0.5 N	+	Parker, 1916	profuse discharge
	distilled water		+		only a few were discharged
	methyl green dye		+		some samples discharged, others did not
	carmine, methylene blue, or *Fundulus* muscle juice	n.g.	−		
	sulfuric acid in sea water	10%	+	Parker and Van Alstyne, 1932	

Hydrozoa n.g	dry gas	n.g.	inhibited	Kepner *et al.*, 1951	specimens bathed in absolute alcohol
	pressure of a falling cover glass plus pressure of a needle		+		
	#1 square, 25 mm coverglass dropped		+		
Chlorohydra viridissima,	acetic acid	0.1 N	+		living hydra in a
Pelmatohydra oligactis,	acetic acid	glacial	+		drop of culture
P. pseudoligactis	chromic acid	1%	+		water on glass
	acetocarmine	n.g.saturated	+		slide—reagent
	carbonic acid	0.1 N	−		added; author did
	potassium hydroxide	0.1 N	+		not specify which
	sodium chloride	40%	+		sp. was used for
	sodium bromide	0.1 N	+		which test
	sodium hydroxide				
	ammonium hydroxide	0.1 N	+		
	mercuric chloride	saturated	+		
	magnesium sulfate	10%	−		
	magnesium carbonate	saturated	−		
	formalin	10%	−		
	cedar oil	n.g.	−		
	oil origanum	n.g.	+		
	I^- in potassium iodide	n.g.	+		
	hydrogen peroxide	3%	−		
	dioxan	n.g.	+		
Hydra attenuata	24 Volts DC	30 μsec	+	Holstein and Tardent, 1984	
Hydra sp.	organic phosphates	1/50,000	+	Lentz, 1966	
	acetylcholine	1/100,000	+		
	decamenthonium	1/10,000	+		
	epinephrine, norepinephrine, histamine	1/10,000	+		
Hydra littoralis	glutathione	n.g.	+	Loomis and Lenhoff, 1956	nonliving material coated with glutathione triggered discharge

Abbreviations: n.g = information not given by authors; + = discharged, − = no discharge; ASW = artificial sea water.

TABLE 2
Compilation of Research on the Discharge of Isolated Cnidae

Class Genus	Isolating Method/Medium	Discharge Agent	Concentration	Discharge +/− (%)	Author(s)
Anthozoa	1 M NaCitrate	distilled water		+	Blanquet and Lenhoff, 1966
Aiptasia					
Anemonia		sea water and HCl	pH 2 or below	+	Pantin, 1942
		food	n.g.	−	
		Na-thioglycollate or trypsin	n.g.	+	Godknecht and Tardent, 1988
Anthopleura	sea water	KCl	0.5 M	+(not complete)	McKay and Anderson, 1988
		$KCl + CaCl_2$	0.5 M, 0.17 M	+(not complete)	
		sea water	pH 2.5	−	
		sea water	pH 10.5	−	
		potassium citrate	100 mM	−	
		taurocholic acid	25 mM	+	
		deoxycholic acid	25 mM	+	
		CHAPS	25 mM	+	
		sucrose in sea water	1 M	−	
		distilled water		−	
		brine shrimp homogenate		−	
		fish skin homogenate		−	
	Ca-free artificial sea water	KCl	0.5 M	−	
		$KCl + CaCl_2$	0.5 M, 0.17 M	+(not complete)	
		sea water	pH 2.5	−	
		sea water	pH 10.5	−	
		potassium citrate	100 mM	−	
		taurocholic acid	25 mM	+	
		deoxycholic acid	25 mM	+	
		CHAPS	25 mM	+	

		sucrose in sea water	1 M	−	
		distilled water		−	
		brine shrimp homogenate		−	
	(1:1) 0.34 M $MgCl_2$/ sea water	KCl	0.5 M	−	
		KCl + $CaCl_2$	0.5 M, 0.17 M	−	
		sea water	pH 2.5	−	
		sea water	pH 10.5	−	
		taurocholic acid	25 mM	+	
		deoxycholic acid	25 mM	+	
		CHAPS	25 mM	+	
		sucrose in sea water	1 M	−	
		distilled water		−	
Anthopleura		Brine shrimp homogenate		−	
Calliactis	Na citrate	EGTA	5 mM	+(95%)	Hidaka and Mariscal, 1988[a]
	ASW	EGTA	5 mM	+(75%)	
	sucrose	EGTA	5 mM	+(40%)	
	distilled water (one drop)	EGTA + sucrose	5 mM	+(50%)	
	Na citrate (1 mM)	EGTA + sucrose	5 mM, 1 mM	+(70%)	
	ASW	EGTA + sucrose	5 mM, 1 mM	+(5%)	
	sucrose (1 mM)	EGTA + sucrose	5 mM, 1 mM	+(4%)	
	distilled water	EGTA + sucrose	5 mM, 1 mM	−	
	1 M glycerol	thioglycollate	50 mM	+(75% after 15 min)	Salleo *et al.*, 1990
Corynactis	smear of oral region	distilled water		+	Robson, 1953 Yanagita and Wada, 1954b
			0.3%	+(100%)	
			0.1%	+(100%)	
		sodium thioglycollate	M/4 pH 8.4	+(100%)	
			M/8	+(100%)	
			M/20	+(100%)	
			M/40	+(100%)	

(Continued on next page)

TABLE 2
Compilation of Research on the Discharge of Isolated Cnidae

Class Genus	Isolating Method/Medium	Discharge Agent	Concentration	Discharge +/− (%)	Author(s)
	1M glycerin	saponin	5%	−	Yanagita, 1960b
		Na-laurylsulfate	>1%	+	
		Na-oleomethyltaurate	>0.1%	+	
		Na-dodecylbenzene	0.001%	+	
		cetyltrimethylammonium bromide	0.01%	+	
		cetylmethylpyridinium bromide	0.01%	+	
		pentaethylene glycol	5.0%	+	
Scyphozoa		thioglycollate	250 mM	+ (100%, few sec)	Salleo *et al.*,
Physalia noctiluca		125 mM	+ (100%, 2 min)		1986
		NaSCN	500 mM	+ (~50% after 15 min)	

[a]All EGTA treatments were for 1 h duration using basitrichous isorhizas.
n.g. = information not given.

that cause discharge. Strong lipid solubilizing agents (CHAPS, taurocholic and deoxycholic acids) have also induced discharge in both isolated and *in-situ* cnidae (McKay and Anderson, 1988). Trypsin and sodium thioglycollate induced significant levels of discharge in isolated nematocysts of *Diadumene* (Yanagita and Wada, 1954). Tardent (1988) experimented with freezing and thawing water and noted that some discharges occurred immediately before ice crystals appeared in the water, and during thawing out. Cnidocytes, placed in distilled water for 10 to 60 min, also caused large numbers of nematocysts to discharge (Tardent, 1988). Recent research has indicated that discharge is a multicellular phenomenon, involving not only the cnidocyte, but surrounding cells and nerves and this cell aggregation has been called the cnidocyte-supporting cell complex (Watson and Hessinger, 1989b) (Figure 4).

C. CNIDOCYTE-SUPPORTING CELL COMPLEX

The cells involved in discharge include the cnidocyte and surrounding cells called supporting cells (Watson and Hessinger, 1988). This complex of cells contains mechanoreceptors and chemoreceptors that "communicate" with the cnidocyte. Based on bimodal and trimodal dose responses, Thorington and Hessinger (1990) surmised that there are at least three populations of cnidocyte-supporting cell complexes that either: 1) respond to tactile stimuli without chemical sensitization, 2) respond only if chemosensitized, or 3) respond to contact with prey vibrating at specific frequencies. Discharge from the complex responding to vibrations was unaffected by inhibitors of calcium channels, but was selectively inhibited by the antibotics gentamycin and streptomycin (Watson and Hessinger, 1994). However, calcium channel inhibitors (nifedipine and verapamil) inhibited discharge for the cell complex that only responds after chemosensitization (Watson and Hessinger, 1994).

In sea anemones, two distinct classes of chemoreceptors located on the supporting cells predispose cnidocytes to discharge nematocysts in the event of prey contact (Thorington and Hessinger, 1988; Watson and Hessinger, 1987). One class of chemoreceptor binds N-acetylated sugars and the other binds certain amino compounds, including proline (Watson and Roberts, 1994). N-acetylated sugars, which occur as conjugates of the surface mucins or external chitin of prey, sensitize cnidocytes to discharge (Thorington and Hessinger, 1988). Glycine and N-acetylneuraminic acid (NANA) have chemosensitized nematocyst discharge in the sea anemone *Aiptasia pallida* (Thorington and Hessinger, 1998). Spirocyst discharge was also modulated by these same compounds as well as proline (Thorington and Hessinger, 1990). Chemoreceptors for N-acetylated sugars may stimulate adenylate cyclase to sensitize and tune mechanoreceptors involved in initiating the discharge of nematocysts (Watson and Hessinger, 1992). Other chemosensitizing agents include glutathione (Loomis and Lenhoff, 1956), glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, acetylcholine (Ach, 1/100,000), and Ach with physostigmine (10^{-5} M, a cholinesterase inhibitor) (Lentz, 1966).

Mechanoreceptors are an important part of discharge; the structure, however, varies among members of Cnidaria. The cnidocytes of Hydrozoans and Scyphozoans feature a hair-like apparatus called the cnidocil, near the margin of the operculum, which functions as a sensory structure for stimulus transduction (Mariscal *et al.*, 1978; Anderson and McKay, 1987). The cnidocil is connected to a network

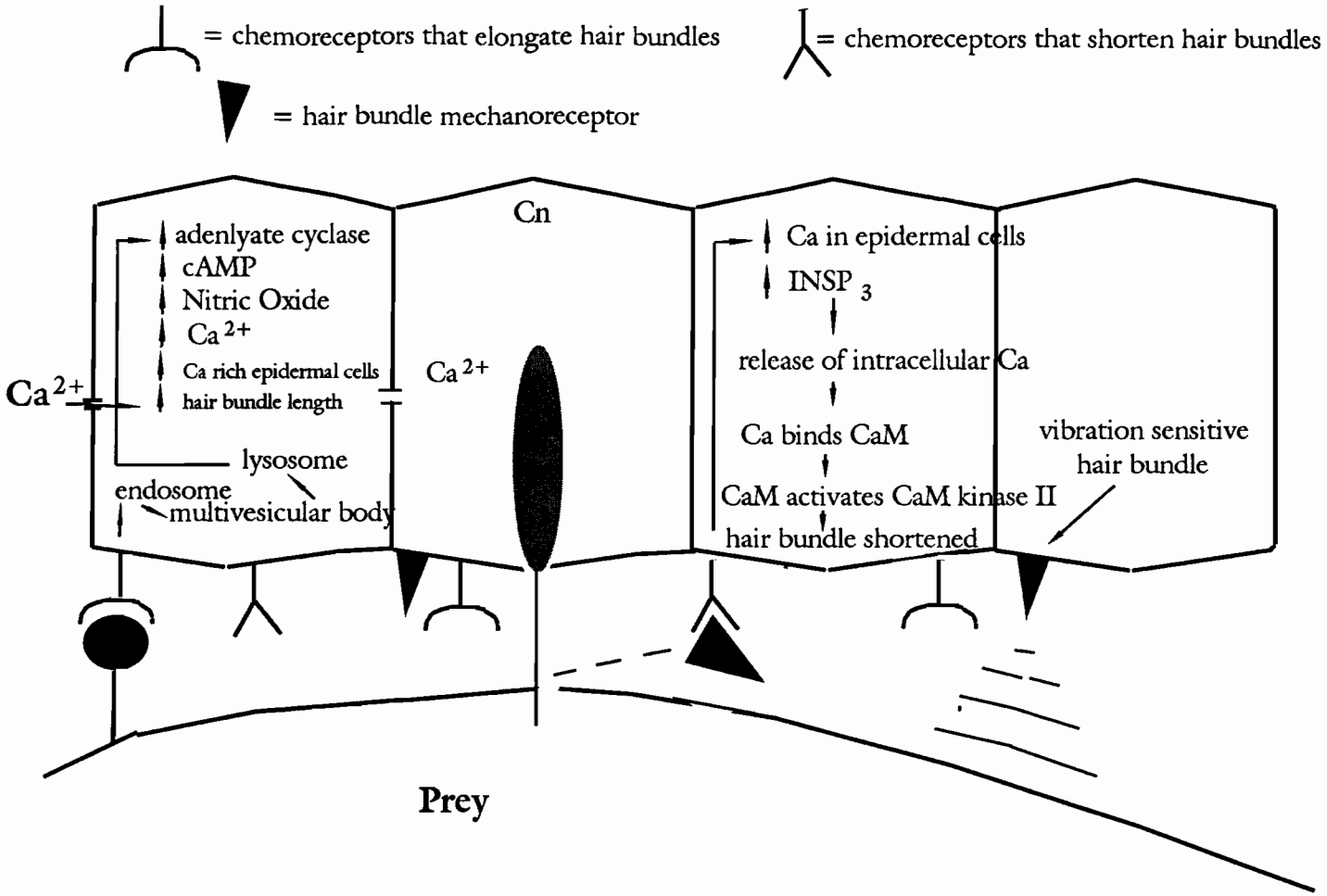

FIGURE 4. Schematic representation of current knowledge regarding the supporting cell complex and cnidae discharge from cnidocytes (Cn). Four receptor types (vibration sensitive, mechano-, and two chemoreceptors) are shown diagramatically. Increases in intracellular adenylate cyclase, cAMP, nitric oxide, calcium ion, and calcium-rich epidermal cells are associated with increased hair bundle length and increased sensitivity to discharge after binding with chemosensitizers. Other receptors respond mechanically to prey or shorten hair bundles through a different set of reactions involving increases in inositol-triphosphate ($INSP_3$) and binding of Ca^{2+} to calmodulin (CaM).

of microfilaments, microtubules, and cross-striated rootlets that support the cnidae near the apical cell membrane of the cnidocyte (Golz, 1994). Some of these microfilaments are composed of actin, a protein known to play a role in converting mechanical deformation into electrical signals via the opening of ion channels (Stidwell *et al.*, 1988). The cnidocil is absent in spirocyts and ptychocysts, which instead feature microvilli or microvilli surrounding a single cilium, respectively (Mariscal *et al.*, 1978). Anthozoan cnidocytes also lack a cnidocil, but have a similar sensory structure called a ciliary cone (Mariscal, 1974).

In Anthozoa, supporting cells adjacent to cnidocytes feature hair bundles (Watson and Hessinger, 1989a). These hair bundles are comprised of a single kinocilium surrounded by a circlet of short microvilli, which is surrounded by stereocilia (Westfall, 1965; Petaya, 1975; Watson and Hessinger, 1991a). Hair bundles elongate after stimulus from N-acetylated sugars, a process dubbed "tuning" (Watson and Hessinger, 1991a; Mire-Thibodeaux and Watson, 1994). Elongation of hair bundles results in nematocysts that discharge at lower vibrating frequencies and amplitudes. Two types of mechanoreceptors, a vibration-sensitive and a contact sensitive mechanoreceptor, have been identified (Watson and Hessinger, 1992).

Receptors for proline are also found on nearby supporting cells rather than the cnidocyte (Watson and Roberts, 1994). An increase of at least 10^{-18} M of proline had the opposite effect of N-acetylated sugars, inducing hair bundles to shorten and tuning nematocysts to fire at higher frequencies which correspond to the increased struggling of the prey, discharging greater numbers of nematocysts into the prey (Watson and Hudson, 1994). This effect was only observed for cells with pre-exposure to N-acetylated sugars (Watson and Hessinger, 1991b; Watson and Hudson, 1994). Glycine and alanine also inhibited NANA-sensitized discharge in *Aiptasia* (Thorington and Hessinger, 1998). The higher discharge threshold produced by the amino acids may also aid in the release of the cnidae from cnidocytes so that prey, once subdued, can move to the mouth of the cnidarian (Thorington and Hessinger, 1998).

D. IONS AND DISCHARGE

Ions can induce discharge, but the effect varies with the type of ion, cnidarian species, and whether the nematocysts are isolated from the cnidocyte. *In-situ* nematocysts discharge at pH values below 4 and above 11, whereas isolated nematocysts discharge at pH values of 2 and below (Blanquet, 1970). Solutions in sea water of such acids as sulfuric (10%) and salts such as potassium bichromate (no concentration given) cause discharge (Parker and Van Alstyne, 1932). Yanagita and Wada (1953) exposed isolated nematocysts from the acontia of *Diadumene* to various acid and alkali solutions. Acids such as sulfuric, citric, hydrochloric, and acetic, with mean pH values ranging from 2.9–3.3 caused 50% of the nematocysts to discharge. Bases such as potassium hydroxide, sodium hydroxide, and ammonium hydroxide, with pH values ranging from 10.8–11.0 caused 50% of the nematocysts to discharge.

Anions have varied in the ability to discharge cnidae. Salleo *et al.* (1983b) noted that the discharge potency of sodium salts isosmotic with seawater followed the lyotropic series: $SO_4^{2-} < CH_3COO^- < F^- < Cl^- < Br^- < NO_3^- < I^- < ClO_4^- < SCN^-$. Santoro and Salleo (1991) also experimented with several different chemical

stimuli that were isosmotic with seawater, including NaSCN, NaCl, NaI, choline chloride, choline iodide, KI, and KCl. They found that artificial seawater with a high K^+ concentration caused a massive nematocyst discharge from *Aiptasia mutabilis*. Among other monovalent cations tested, NH_4^+ also stimulated *in-situ* cnidae discharge (Yanagita, 1960a). However, Na^+ and Li^+ as chloride salts were not effective (Yanagita, 1960a). Potassium was a key ion for discharge of nematocysts of *Metridium* (Parker, 1905), *Diadumene luciae* (Yanagita, 1960a), *Anthopleura elegantissima* (McKay and Anderson, 1988), *Tubularia mesembryanthemum* (Kawaii *et al.*, 1997), and *Aiptasia pallida* (Blanquet, 1970). This effect was muted in isolated nematocysts (Yanagita, 1959a; Blanquet, 1970; McKay and Anderson, 1988). The effect of potassium was also dependent on the presence of calcium (McKay and Anderson, 1988). Blanquet (1970) found that discharge of *in-situ* nematocysts was induced by several different potassium salts at concentrations isosmotic with seawater or half that. Chloride salts (with the exception of isolated discharge in full strength NaCl) and divalent cations such as Ca^{+2} and Mg^{+2} did not induce discharge. Drops in chloride ion concentrations increased the reaction time of *in-situ* anemone nematocysts (Yanagita, 1960b). Sodium ion depletion has also been effective in blocking discharge (Brinkman *et al.*, 1996). For isolated cnidae of *Diadumene*, natural seawater was effective in firing nearly 100% (Yanagita, 1959a).

Calcium plays a key role in the discharge of some cnidae. An increase in free Ca^{2+} in *Tubularia* cnidocytes just prior to discharge was noted by Kawaii *et al.* (1997). Lubbock *et al.* (1981) found very high concentrations of calcium in the fluid of nematocyst capsules (500–600 mM/kg wet weight) compared to concentrations in the cnidocyte (9–18 mM/kg) or seawater (7 mM/kg). However, the high intracapsular calcium concentrations were conspicuously absent from immature nematocysts. This corresponds to data which showed that discharge of nematocysts can be age-dependent (Kawaii *et al.*, 1997). After discharge, the ionic composition and dry mass inside the capsule changes dramatically and becomes similar to the surrounding seawater (Lubbock *et al.*, 1981; Lubbock and Amos, 1981).

The removal of calcium from the medium has inhibited discharge of *in-situ* nematocysts (Santoro and Salleo, 1991; Kawaii *et al.*, 1997). Similarly, reduction of Ca^{2+} below 1 mM decreased discharge of *in-situ* nematocysts of *Haliplanella luciae* by 80% in the presence of proline (Russell and Watson, 1995). Conversely, an increase in intracellular calcium by the addition of 10^{-8} M thapsigargin, an inhibitor of Ca^{2+}-ATPases that store Ca^{2+} into vesicles, increased discharge (Russell and Watson, 1995). In contrast to *in-situ* nematocysts, removal of calcium in isolated nematocysts has induced discharge. For example, Lubbock and Amos (1981) used potassium citrate to chelate calcium; the drop in calcium induced discharge in nematocysts isolated from the anemones *Rhodactis rhodostoma* and *Anthopleura elegantissima*. This effect was reversible by the addition of $CaCl_2$, but not NaCl, KCl, or $MgCl_2$. Salleo *et al.* (1983a) observed that isolated nematocysts of *Pelagia noctiluca* pretreated with $CaCl_2$ were inhibited from discharging when exposed to trypsin, an effect not observed in controls or for nematocysts treated with $MgCl_2$. Hidaka and Mariscal (1988) noted similar results using isolated nematocysts from *Calliactis tricolor*, but discharge depended on the medium used for isolation; *e.g.*, no discharge occurred in Ca^{2+}-free artificial seawater, but there was heavy discharge among nematocysts isolated with sodium citrate and subsequently treated with low-osmolarity solutions. Hidaka and Mariscal (1988) surmised that the loss of calcium from the capsule augmented discharge.

The effect of calcium ion does not apply to all cnidae. Salleo *et al.* (1990) noted that very little calcium was discharged from the nematocysts of *Calliactis parasitica*. Salleo *et al.* (1990) also found that Ca^{2+} and Mg^{2+}-free artificial seawater did not induce discharge of isolated nematocysts. Similarly, Mariscal (1984) has observed holotrichous isorhiza nematocysts of *Haliplanella luciae* that appear to lack calcium, suggesting that other ions and chemical messengers aid in discharge.

Cnidocyte cytoplasm was higher in potassium, chloride, and sulfur than in the resting nematocyst capsule, indicating that an ionic gradient is maintained between the two fluids (Lubbock *et al.*, 1981). The gradient is likely maintained by the capsule wall membrane, since the capsule wall has been shown to be permeable to dyes as large as 670–700 Daltons (Lubbock and Amos, 1981; Tardent, 1988).

E. INHIBITION OF DISCHARGE

In addition to the effects of ion depletion as noted above, other compounds have been observed to inhibit discharge. Salleo *et al.* (1994) treated *in-situ* nematocysts of *Pelagia noctiluca* with 50 Fmol/L gadolinium applied through a gelatin probe and found a significant decrease in the number of discharged nematocysts in comparison with untreated cnidae. Streptomycin was found to block nematocyst discharge of *Hydra vulgaris* (Gitter *et al.*, 1993) and *Stauridiosarsia producta* (Brinkman *et al.*, 1996). Dry gas (Parker and Van Alstyne, 1932), urethane (5%), and Na-diethylbarbiturate (1%) in seawater were also found to prevent extrusion of nematocysts (Yanagita, 1959b). Divalent cations can also inhibit discharge. For example, Parker (1916) found that magnesium sulfate suppressed discharge in *Metridium*. Yanagita (1959b, 1960a) found that Mg^{2+} inhibited discharge of *in-situ* nematocysts of *Diadumene luciae*. Salleo *et al.* (1984) found that Ca^{2+}, Mg^{2+}, and Ba^{2+} inhibited the discharge effects of iodide on isolated nematocysts of *Pelagia noctiluca*.

F. NERVES, ELECTRICITY, AND DISCHARGE

The primitive nervous system of cnidarians may modulate the responsiveness of cnidocytes in some species (Wagner, 1905; Thorington and Hessinger, 1988). For example, several species of *Hydra* fed to repletion lose their ability to discharge nematocysts (Smith *et al.*, 1974; Ruch and Cook, 1984; Grosvenor and Kass-Simon, 1987). A similar suppression of discharge after feeding was observed in the anemone *Calliactis tricolor* (Sandberg *et al.*, 1971). Even neighboring hydranths that were unfed became inactive when nearby hydranths were fed (Clark and Cook, 1986). This effect was attributed to the discharge products of the nematocysts, rather than from the prey or depletion of nematocysts *per se*.

Millot (1968, cited by Thorington and Hessinger (1988)) found that varying intensities of light had some effect on cnidocytes as well. Millot (1968) observed that complete darkness and bright light (*e.g.*, 40 klx) depressed the responsiveness of cnidocytes to tactile stimulation, and totally inhibited chemosensitization thereby preventing capture of natural prey. Millot found that prey capture was optimal in intermediate light intensities (*e.g.*, 4–6 klx).

The effect of exposing cnidocytes to varying intensities of light as discussed above and of feeding *Hydra* to repletion indicate that the nervous system may play a role in regulating discharge. Ultrastructural observations have shown a physical connection between nerve cells and cnidocytes (Hufnagel and Kass-Simon, 1988). Lentz (1966) observed that neurohumoral agents such as acetylcholine, 5-hydroxytryptamine, epinephrine, and histamine chemosensitized *Hydra* cnidocytes to discharge. Norepinephrine was especially effective for atrichous isorhizas. However, the nematocysts may fire independently of the nerve cells (Lentz, 1966; Aerne *et al.*, 1991).

Direct measurement of electrical activity *in situ* has shown that electrical activity is associated with discharge. For example, Lubbock and Shelton (1981) used *Anthopleura elegantissima* and showed that discharge was associated with a triphasic spike in voltage up to 50 μV in amplitude and about 350 msec in duration. It was not clear if the spike in voltage caused the discharge or was a consequence of it. There were also a variable number of pulses, up to 10 μV and up to 50 msec, which preceded the spike. Some of these pulses were also induced by contact with materials that did not induce discharge. McKay and Anderson (1988) noted currents both into and out of cnidocytes, observing that ion flow across the membrane was voltage dependent. Brinkman *et al.* (1996) noted electrical impulses that were associated with mechanical stimulation of the cnidocil apparatus.

Electricity can stimulate discharge of *in-situ* cnidae, but it has not been effective for isolated cnidae, such as the nematocysts of *Metridium* (Glaser and Sparrow, 1909). This effect was later corroborated by Parker and van Alstyne (1932) for *Metridium* and *Physalia*, and by Yanagita (1960a) for *Diadumene*. Pantin (1942) and Yanagita (1960a) both observed that the electrical effects were local and not propogated throughout the acontial filament. The threshold direct current Yanagita (1960a) needed to induce extrusion varied from 8 to 20 μA (delivered by a 67.5 V battery through a 100 kΩ potentiometer). Pantin (1942) experimented with *Anemonia sulcata*, and found that electrical shocks (240 V from a 2 μF condenser and passed through a 400 Ω potentiometer) stimulated cnidae discharge. Anderson and McKay (1987) found that isolated nematocysts of *Cladonema* and *Chrysaora* failed to discharge after electrical stimulation, despite detection of a variety of voltage-dependent ionic currents. *In-situ* nematocysts of *Physalia* similarly failed to discharge (Anderson and McKay, 1987). In hydra tentacles, stenotele and desmoneme nematocysts readily discharged using 24 V DC, but the isorhizas did not (Tardent, 1988).

G. INTRA- AND INTERCELLULAR SIGNALING

Detailed knowledge of the intra- and intercellular chemical signaling that induces discharge remains to be discovered. It is known that channels in membranes control the flow of ions, and in turn, these channels are controlled by various means (Ashcroft, 2000). For example, flow of Na^+ across a voltage-gated sodium channel is dependent on the electrical potential across the membrane which is a function of the ion concentrations. Other channel gates are controlled by ligands such as the neurotransmitters glycine and acetylcholine (Ashcroft, 2000). When the ligand binds to its receptor, the gate opens, resulting in ion flow.

In cnidocytes and the supporting cells, it is likely that these channels play a role in signaling the nematocyst. Watson and Hessinger (1992) noted that receptors for N-acetylated sugars stimulated adenylate cyclase which increased intracellular cAMP. These authors found that agents which increase cAMP (dibutyryl cAMP, forskolin, and cholera toxin) sensitized contact-sensitive receptors and vibration-sensitive receptors. Later work (Watson and Hessinger, 1994) suggested the involvement of calcium channels after the receptor for the NANA was activated. Watson and Hessinger (1987) observed that bovine submaxillary mucin first attached to supporting cell chemoreceptors, then was internalized into endosomes and translocated to multivesicular bodies and lysosomes. Nitric oxide (NO) was produced by supporting cells of *Aiptasia diaphana* and discharge was induced *in situ* after perfusion with NO (Salleo *et al.*, 1996). Calmodulin was also shown to be part of the intracellular signaling after chemosensitization since a calmodulin inhibitor inhibited discharge (Russell and Watson, 1995). Calmodulin inhibition has also prevented the protozoan *Giardia lamblia* from emerging from its cyst (Bernal *et al.*, 1998). Mire-Thibodeaux and Watson (1993) noted that after chemosentization with NANA, rare epidermal cells that are rich in calcium increase two- to three-fold. These cells are high in calcium with or without sensitization. Proline has been observed to increase intracellular calcium in epidermal cells (Russell and Watson, 1995). Inhibitors of L-type calcium channels (verapamil and nifedipine) also inhibited discharge of nematocysts sensitized by NANA, but not proline (Russell and Watson, 1995). Inhibitors of certain intracellular calcium channels (ryanodine and procaine) inhibited discharge sensitized by proline, but not by NANA. Lubbock *et al.* (1981) suggested that substances which unbind calcium are released from the cnidocyte cytoplasm and act in concert with the capsule migration to the cell membrane to effect discharge. Under alkaline conditions, the ionophore exchanges protons in the sporoplasm with extracellular cations. As protons are lost, the pH increases, creating a proton gradient between the sporoplasm and the polar capsule. Cations are then exchanged for protons, creating an osmotic imbalance. Water movement into the polar capsule results in rapid swelling, causing extrusion of the polar filament.

IV. MYXOZOAN POLAR FILAMENT DISCHARGE

A. POLAR CAPSULE STRUCTURE

One of the principal characteristics of the phylum Myxozoa is the presence of polar capsules in both the myxosporean and actinosporean stages. Numerous studies have reported on the ultrastructure and development of polar capsules (Lom and Puytorac, 1965; Lom and Vávra, 1965; Lom, 1969; Jensen and Wellings, 1972; Stehr, 1986; Lom and Dyková, 1988, 1992), so development will not be discussed here.

The basic structure of polar capsules is simpler than for most cnidae. The polar capsule is composed primarily of a thick capsular wall, an eversible polar filament that is contiguous with the wall, and a "stopper" of unknown composition that covers the lumen of the inverted filament. The filament is hollow and spirally twisted along its length (Lom and Dyková, 1992). The everted filament is sticky and aids in attachment to the host. Variation in the coils of the filament within the capsule and filament length are characteristics that aid identification (Fantham *et al.*, 1939; Lom

and Dyková, 1992) (Figure 4). In some species, such as *Myxobolus subcircularis*, filament length can reach about 10 times the length of the myxospore and up to 117 μm (Fantham *et al.*, 1939). The polar capsule wall has two layers; the inner wall is electron lucent and resistant to alkaline hydrolysis and the outer wall is proteinaceous (Lom and Dyková, 1992).

B. POLAR FILAMENT DISCHARGE

The potential of premature polar filament discharge to disarm myxozoan parasites has long been recognized (Hoffman *et al.*, 1965). However, research on extrusion of polar filaments is limited. Since the two-host life cycle for myxozoans was not demonstrated until 1984 (Wolf and Markiw, 1984), most of the data summarized here was derived from work with the myxospore stage. Gurley (1894) observed a small portion of alcohol-fixed myxospores extrude polar filaments when sulfuric acid was combined with iodine water. Sulfuric acid by itself did not give similar results with *Henneguya rupestris*; nor did hydrochloric acid (Herrick, 1941). A failure of acids to extrude filaments has also been noted for *Myxobolus cerebralis* (Uspenskaya, 1957) and *M. cartilaginis* (Hoffman *et al.*, 1965). However, Herrick (1936) reported the extrusion of some *M. kostiri* filaments after using concentrated sulfuric acid.

Stomach acids were evaluated by a few researchers using various approaches. For example, Uspenskaya (1957) put a filter bag containing myxospores in a trout stomach (species not given); upon retrieval 56 h later, no myxospores had discharged. Plehn (see Uspenskaya, 1957) similarly noted no extrusion after 24–48 h. The stomach enzyme trypsin was evaluated by Hoffman *et al.* (1965) with sodium bicarbonate with negative results. However, Iversen (1954) used the digestive juices of a freshly killed rainbow trout and was successful in extruding polar filaments of *Myxobolus squamalis*. Using *Myxobolus muelleri* spores, Lom (1964) was unable to extrude filaments using trypsin or pepsin in varying concentrations and varying pH; sodium thioglycollate in concentrations up to 4 M and extreme pHs were also unsuccessful. Davies (1968) observed that most of the spores of *Henneguya zschokkei* were discharged in 5% formalin, whereas a tailless form did not discharge.

The other end of the pH spectrum has been more successful for polar filament discharge. Potassium and sodium hydroxide at concentrations of greater than 1–2% extruded polar filaments in *Myxidium minteri*, *Chloromyxum majori*, *Myxobolus cerebralis*, *M. kisutchi*, *M. notemigoni*, and *M. cartilaginis* (Yasutake and Wood, 1957; Uspenskaya, 1957; Lewis and Summerfelt, 1964; Hoffman *et al.*, 1965). The effect of potassium hydroxide (KOH) on discharge of the polar filaments was temperature dependent: using 1.5% KOH, Hoffman *et al.* (1965) noted that no filaments of *M. cartilaginis* myxospores fired at 6°C, but at 23 and 40°C, 57% and 96% of polar filaments were extruded. Herrick (1936) used ammonium hydroxide (no concentration or duration noted), but was unsuccessful in extruding polar filaments of *Myxobolus kostiri*. Herrick (1941) was similarly unsuccessful using potassium hydroxide on *Henneguya rupestris*.

Other materials have been experimented with, most unsuccessfully. Hoffman *et al.* (1965) failed to extrude polar filaments of *Myxobolus cartilaginis* using a variety of detergents at various concentrations, oxidizers (10% hydrogen peroxide or 5% potassium permanganate), a chelating agent (Versene), acetone, malachite green,

quinine sulfate, sodium ethyl mercurithio-salicylate, calomel, carbasone, formalin, Furazolidone, and pyridyl-mercuric acetate. However, these authors were successful extruding a small percentage using 0.5% alkyl dimethyl-benzyl-ammonium chloride, sodium hypochlorite, and 10% calcium cyanamide. Another successful discharge agent was potassium hydrate, used on *Myxobolus discrepans* (Kudo, 1930). Using urea (saturated solution at 23EC), Lom (1964) was able to induce extrusion after 30 min to 2 h in *Myxobolus*, *Thelohanellus*, *Henneguya*, *Myxidium*, *Zschokella*, *Chloromyxum*, and *Sphaeromyxa*. Weaker solutions were much less effective.

Work with hydrogen peroxide indicated that it could induce discharge of polar filaments at high concentrations. For instance, approximately 1.5% hydrogen peroxide caused only a few filaments of the microsporidian *Nosema bombycis* to discharge, whereas 15–20% solutions discharged a "great many" filaments (Kudo, 1918). Lom (1964) reported that 30% hydrogen peroxide caused 100% of the filaments of *Myxobolus muelleri* to extrude, but lower concentrations failed to induce 100% extrusion; he further noted that it was ineffective for many species of myxosporeans. Kudo (1918) reported that hydrogen peroxide discharged filaments of myxospores of *Myxobolus funduli*, *Zschokkella acheilognathi*, and *Myxidium* sp. as well. Hoffman *et al.* (1965) failed to extrude polar filaments of *M. cartilaginis* after 30 min using 10% hydrogen peroxide.

Some researchers have experimented with water, dessication, and salinity. Ward (1919) reported extruding polar filaments of *Myxobolus auratus* after spores were in "plain water" for over 24 h. Distilled water at 39.5°C for 60 min did not work for *M. cartilaginis* (Hoffman *et al.*, 1965). Herrick (1941) extruded a few filaments using 0.85% saline. If a saline solution of spores was dried on a slide and distilled water added, myxospores of *Myxobolus osburni* discharged the polar filaments (Herrick, 1936). However, dessication followed by the addition of distilled water failed to discharge filaments of *M. kostiri* (Herrick, 1936). Drying followed by wetting was also unsuccessful for *Henneguya rupestris* (Herrick, 1941). Kudo (1918) noted that hydrogen peroxide failed to extrude polar filaments of spores that had been dried, whereas fresh spores would respond. This would indicate that the spores were killed by drying and the polar capsules were inactivated. This would also indicate that the ability to discharge filaments may be a potential means of assessing viability of spores.

Yokoyama *et al.* (1995) was able to discharge filaments of raabeia-type actinospores of *Myxobolus cultus* using mucus from a variety of fish species, including goldfish *Carassius auratus*, common carp *Cyprinus carpio*, loach *Misgurnus anguillicaudatus*, rainbow trout, catfish *Parasiluris asotus*, and Japanese eel *Anguilla japonica*. Yokoyama *et al.* (1995) also discharged filaments with mucin from bovine submaxillary gland. Conversely, El-Matbouli *et al.* (1999) failed to extrude polar filaments of *Myxobolus cerebralis* triactinomyxons using rainbow trout mucus. Uspenskaya (1995) was also able to discharge filaments of *Zschokkella nova* using mucus of goldfish.

Xiao and Desser (2000) observed polar filament extrusion and ameboid movement of the sporoplasms of actinospores after 1–2 min in a mix of lake water and mucus. The extrusion of aurantiactinomyxon, neoactinomyxum, echinactinomyxon, and raabeia forms was induced by mucus from a variety of species including brown bullhead *Ameiurus nebulosus*, yellow perch *Perca flavescens*, pumpkinseed *Lepomis gibbosus*, creek chub *Semotilus atromaculatus*, golden shiner *Notemigonus crysoleucas*, common shiner *Luxilus cornutus*, white sucker *Catostomus commersoni*, and

fathead minnow *Pimephales promelas*. The percentage of extruded polar filaments varied with species and actinospore, and no actinospore was species specific. However, Xiao and Desser (2000) noted that some triactinomyxon forms were discharged by mucus from a narrower range of species. For example, in the "triactinomyxon C" form, discharge was induced in >90% of the actinospores by common shiner, fathead minnow, and golden shiner mucus, but was 12% or less in the others, including the other cyprinid (creek chub) (Xiao and Desser, 1998). Triactinomyxon "F" had an even narrower range, discharging 77% in mucus of common shiner, 12% in golden shiner, and 8% or less in the remaining species. Recent work with mucus has shown that it contains compounds which can alter membrane permeability (Ebran *et al.*, 2000). This may be a part of the signaling mechanism for those Myxozoa that respond to mucus.

V. COMPARISON OF CNIDAE AND POLAR CAPSULES

A. SIMILARITIES

The early development of polar capsules and cnidae are very similar, suggesting a common ancestry (Slaughterback and Fawcett, 1959; Lom and de Puytorac, 1965; Lom, 1969). Polar capsules and cnidae arise from specialized cells and contain a coiled filament continuous with the capsular wall (Lom, 1989). In both structures, the filament is coiled when encysted, and uncoils during discharge (Skaer and Picken, 1965; Lom, 1989). Cnidarian spirocysts and ptychocysts are similar to Myxozoa in that the long filament is sticky after everting (Barnes *et al.*, 1993; Lom and Dyková, 1992). However, in Myxozoa that have been examined, the hollow filament is solid at the tip, functioning as an anchoring mechanism rather than a conduit (Lom and Dyková, 1992).

The Myxozoan polar capsule perhaps most resembles the desmoneme form of nematocyst. The size of the desmoneme nematocyst (about 9 μm long) and the polar capsules (generally 5–7 μm) are similar, and the filament has no barbs. Ptychocysts are also similar to polar capsules, featuring a long filament with a closed tip and no spines; however, ptychocysts are larger in size (29–75 μm long) (Mariscal *et al.*, 1977a). Siddall *et al.* (1995) suggested that polar capsules were "typical nematocysts bearing atrichous isorhiza" which lack spines.

Siddall *et al.* (1995) noted that the actinospore stage of many myxozoans exhibit radial symmetry, similar to cnidarians. For example, triradial symmetry occurs in triactinomyxons, spheaeractinomyxons, auractinomyxons, and echinoactinomyxons, and up to octameric symmetry occurs in tetractinomyxons, hexactinomyxons, and synactinomyxons. Mature myxospores of the order Multivalvulida, *e.g. Kudoa* sp., also have radial symmetry (Lom, 1989).

Both polar capsules and cnidae appear to respond to pressure (Kepner *et al.*, 1951; Guilford, 1963). Isolated nematocysts can discharge, indicating that its intracapsular pressure is independent of the cnidocyte (Tardent, 1988). Pressure from a falling cover slip causes discharge of Hydrozoan cnidae (Kepner *et al.*, 1951) and pressure on wet mounts induces extrusion of polar filaments of *Henneguya doori* (Guilford, 1963), *H. rupestris* (Herrick, 1941), *Myxobolus neurophila* (Guilford, 1963),

and *M. squamalis* (Iversen, 1954). Release of pressure within the capsule is probably part of the discharge mechanism for both phyla.

Another similarity between cnidae and polar capsules is the "stopper" or "lid" that caps the inverted filament. In theory, one of the components disintegrates by a certain "trigger" process, and the tension in the capsule is released, firing the filament. Tardent (1988), using high-speed cinematography, noted that opening of the tri-partate cap of a hydra stenotele was the first detectable reaction in the discharge process. The tri-partate apical flaps are also a feature of some Anthozoan nematocyst capsule tips (Watson and Mariscal, 1985). The "stopper" of cnidae is thought to be composed partially of keratin (Brown, 1950; Yanagita and Wada, 1954).

The electron-dense "stopper" has been described in Myxozoa, but the composition of this structure is unknown (Lom and de Puytorac, 1965). The lack of discharge after treatment with digestive enzymes (Uspenskaya, 1957) suggests that the cap is made of a resistant compound. Keratin, mucins, spongin, chonchiolin, and low molecular weight peptides are reported to be resistant to the effects of pepsin, the most common digestive enzyme (Fänge and Grove, 1979). Lack of discharge may also indicate that destruction of the "stopper" protein does not automatically infer discharge, as observed by Uspenskaya (1982). Lom and Dyková (1992) noted that there was little difference in the structure of polar capsules between myxospore and actinospore, except for the capsule tip. In actinospores, the stopper mechanism is a granular cone that is occasionally covered with microtubules that cover the capsulogenic cell membrane and stick into the aperture between the sutural edges. In myxospores, the canal for filament extrusion is filled with a projection. These differences may be indicative of different stimuli required for each stage. There is also some variation in the ultrastructure of myxospore polar capsules among genera such as Zschokella, Fabespora, and Sphaerospora (Lom and Dyková, 1992), that may indicate some variation in stimuli required for discharge.

Both cnidae and polar capsules respond to treatment with strong acids and bases (Jones, 1947; Yanagita and Wada, 1953; Lewis and Summerfelt, 1964; Hoffman *et al.*, 1965). Unfortunately, pH, durations, temperatures, and concentrations were not reported in many of these studies, making it difficult to ascertain genetically based differences in susceptibility to acids and bases among myxozoans and cnidarians. Wagner *et al.* (2002) observed that discharge of triactinomyxon polar filaments of *Myxobolus cerebralis* markedly increased as pH rose from 11.2 to 12.9 (100% discharge). This was slightly higher than the pH levels noted by Blanquet (1970) who noted a sharp increase in discharge of isolated anemone cnidae if seawater pH increased from 9.7 to 11. Jones (1947) noted a high level of discharge of hydra cnidae using 0.1 N NaOH or NH_4OH, but no pH level was given.

Potassium is a key ion for the discharge of cnidae (Blanquet, 1970) and polar filaments of Microsporidia (Undeen, 1978) and Myxozoa (Gurley, 1894; Wagner *et al.*, 2002). Concentrations of K^+ that induced discharge varied among the cnidarians studied, due in part to whether cnidae were isolated or *in-situ* and the developmental stage. For example, Blanquet (1970) discharged *in-situ* cnidae using 0.28–0.56 M KCl, but discharge of isolated cnidae was only about 9% at 0.56 M KCl. Blanquet (1970) diluted the KCl solutions with distilled water and noted that isolated cnidae discharge increased to 90% as the concentration decreased to 35% of the original concentration (about 0.2 M). Yanagita (1960a) increased discharge of *in-situ* cnidae

of *Diadumene* as concentrations increased from 10 to 500 mM of KCl or K_2SO_4 mixed with NaCl, whereas isolated cnidae failed to respond. Similarly, McKay and Anderson (1988) observed that *in-situ* cnidae were discharged by 0.5 M KCl in seawater, whereas isolated cnidae only occasionally responded to the same concentration. Species differences in sensitivity were noted by Pantin (1942); 30 mM KCl in seawater discharged *Calliactis* cnidae, whereas *Anemonia* cnidae required 120–240 mM KCl to discharge. *Myxobolus cerebralis* triactinomyxons discharged in response to KCl concentrations that were similar (0.16 to 1.32 M) to those noted above for Cnidaria (Wagner *et al.*, 2002). Potassium hydroxide at concentrations of greater than 1–2% extruded polar filaments in several species of myxozoans (Yasutake and Wood, 1957; Uspenskaya, 1957; Lewis and Summerfelt, 1964; Hoffman *et al.*, 1965), but it is not clear how much of the effect is due to high pH rather than K^+ *per se*. McKay and Anderson (1988) showed that potassium channels which regulate intercellular movement of ions are part of the chemical signaling involved in cnidae discharge; a K^+-channel blocker (4-aminopyridine) inhibited discharge when mixed with KCl.

The similarities noted above have led some researchers to use modern genetic analysis to better understand phylogenetic relationships between Myxozoa and Cnidaria. Smothers *et al.* (1994) compared 18S ribosomal RNA sequences from a variety of metazoan and protozoan organisms and found that myxozoan representatives from *Henneguya* and *Myxobolus* were more closely allied with Nematoda and other metazoans than they were with Cnidaria, Porifera, and protozoans. However, Siddall *et al.* (1995) examined 18S rDNA and grouped *Henneguya* and *Myxobolus* with the cnidarian *Polypodium*.

B. DIFFERENCES

There are a number of fundamental differences between the two phyla. Myxozoa typically alternate between parasitic myxosporean and actinosporean stages (Wolf and Markiw, 1984; Uspenskaya, 1995; Székely *et al.*, 1998), whereas cnidarian life-cycle stages include the sedentary polyp or hydranth, free medusa, and planula larvae (Storer *et al.*, 1972). While both cnidae and polar capsules are organelles within a specialized cell, early development in Myxozoa differs in that one generative cell envelopes the other, the outer cell protecting the further mitosis and development of the inner cell (Lom, 1969, 1989). This envelopment process does not occur in Cnidaria (Slautterback and Faucett, 1959). Another fundamental difference between the two phyla is the cellular organization; cnidarians characteristically have two cell layers, the ectodermis and gastrodermis, with a mesoglea between the two layers (Storer *et al.*, 1972). This level of organization is not seen in Myzozoa.

Multicellular involvement in discharge has been demonstrated in Cnidaria, however, in Myxozoa it is not known if or how the polar capsules are influenced by the capsulogenic cell or other neighboring cells. Evidence against multicellular involvement in Myxozoa was presented by Yokoyama *et al.* (1995) who reported that *Myxobolus cultus* actinospores failed to respond to some amino acids and NANA. Using the cnidarian chemosensitizers NANA and proline, Wagner *et al.* (2002) was also unable to induce significant discharge of polar filaments of *Myxobolus cerebralis* triactinomyxons. If polar filament discharge is dependent on intercellular signaling,

the chemosensitizing ligands appear to be different compounds than those that work for Cnidaria. Alternately, discharge in Myxozoa may be a unicellular phenomenon. In this scenario, polar capsules should respond more like isolated cnidae than *in-situ* cnidae. Evidence for this is evident in the comparison of the effects of pH on discharge. Myxozoa responded to pH levels that were more extreme than for cnidae *in-situ* (Blanquet, 1970). Isolated nematocysts do not discharge until pH drops to 2 or below (Blanquet, 1970). Some Myxozoa have discharged after exposure to strong acids (*e.g.*, *Myxobolus kostiri*) (Herrick, 1936), while others such as *Myxobolus kisutchi* and *M. cerebralis* have not (Yasutake and Wood, 1957; Uspenskaya, 1957). Unfortunately, in the myxozoan references, the exact pH, duration of exposure, and concentration were not reported. In tests performed by Wagner *et al.* (2002), in which a pH of 1.1 was required to significantly increase discharge of *Myxobolus cerebralis* polar filaments, results supported the hypothesis that polar capsules respond more like isolated cnidae. Contrary to results with isolated cnidae (Salleo *et al.*, 1983a), trypsin has not been an effective discharge agent for Myxozoa (Uspenskaya, 1957), indicating different discharge mechanisms.

Discharge of isolated cnidae has been induced principally by compounds that alter the ionic concentration (*e.g.*, distilled water, 1 M glycerin, and 1 M sodium citrate) (Blanquet, 1970) or membrane integrity (lipid solubilizing agents, thioglycollate, trypsin) (Yanagita and Wada, 1954; McKay and Anderson, 1988), but not human hair, skin or other ligands (Yanagita, 1960a). For example, calcium removal induced discharge of isolated cnidae (Lubbock and Amos, 1981; Hidaka and Mariscal, 1988). Tests of calcium removal with myxozoans have been conflicting. Uspenskaya (1982) observed that removal of calcium from the external environment with 2 mM EGTA (no pH or duration given) induced 100% discharge in a variety of Myxosporidia. Using 25 mM of the calcium chelators EGTA or sodium citrate, Wagner *et al.* (2002) failed to extrude polar filaments of *Myxobolus cerebralis* triactinomyxons after 1 h at rates significantly above that observed for pH 9.4 alone.

Another difference between Myxozoa and Cnidaria is the presence of a primitive nerve network in cnidarians that is absent in myxozoans. Neurochemicals such as acetylcholine induced cnidae discharge (Lentz, 1966), but the neurochemicals acetylcholine chloride, angiotensin, and bradykinin failed to extrude polar capsule filaments in *Myxobolus cerebralis* actinospores at similar concentrations (Wagner *et al.*, 2002).

Electricity has induced both cnidae and polar filament discharge, but threshold voltages differ. Wagner *et al.* (2002) induced polar filaments of *Myxobolus cerebralis* to discharge, but at much higher voltages of direct current (3 kV for 99 μsec) than used for cnidae extrusion (24–67.5 V) (Yanagita, 1960a; Tardent, 1988). Interpretation of the mechanism for electrical discharge is complicated by some of the "side-effects" of electrical treatment of water which can include formation of free oxygen and hydrogen, hydroxyl and hydroperoxyl radicals (producing hydrogen peroxide), addition of metal ions from the electrodes, a temperature increase, or physical shock waves (Palaniappan and Sastry, 1990). For cnidarians, the effect of electricity is limited to *in-situ* cnidae and does not work on all types of cnidae (Glaser and Sparrow, 1909; Anderson and McKay, 1987; Tardent, 1988). This phenomenon is more evidence for the ionic intercellular signaling controlled by voltage-gated membrane channels that are artificially stimulated by external electrical potentials.

In summary, there are a number of similarities and differences between cnidae and polar capsules. Similarities include early development, radial symmetry, helically coiled filaments that are sticky develop within specialized cells and are capped with an electron dense "stopper," response to pressure, and sensitivity to K^+, electricity, and extreme pH values. Myxozoans differ from cnidarians in the lack of a nerve net, less cellular organization, different life-cycle stages, and in the response to chemosensitizers, neurochemicals, and Ca^{2+} removal.

Clearly, more work remains to be done, especially for Myxozoa. For example, does premature polar filament extrusion prevent infection? What are the natural chemical and physical triggers for actinospore discharge? How do these triggers differ in the myxosporean stage or among different species? Hopefully, these data presented will provide the necessary background information to inspire additional research.

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