

The Chemical Composition of Sea Nettle Nematocysts* (34113)

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Interest in the stinging apparatus of the sea nettle (*Chrysaora quinquecirrha*) has been stimulated recently because of the economic problem produced by this organism. Resort activity is severely curtailed during the summer season when the nettle is prevalent. The nettle's toxic activity is located in the nematocyst and appears to be membrane-bound (1). The chemical composition of sea nettle nematocyst suspensions was determined in order to obtain data concerning the structure of the organelle and the pathogenesis of the sting.

Materials and Methods. Adult stages of *Chrysaora quinquecirrha* were obtained from the Chesapeake Bay during August and September, 1968. Whole tentacles (WT) and nematocyst suspensions (NS) were prepared as described earlier (1).

The following chemical determinations were performed on freeze-dried specimens according to standard techniques:

N₂ (2), Cl (3), C (4), H (4), S (4), O (4), ash (4), P (5), DNA (6), RNA (7), reducing sugars (8), sialic acid (9), glycerol (10), hexosamine (11), saponifiable fats (12), and cholesterol (13).

Preliminary experiments were done to determine the conditions for optimal yield of these substances from the jellyfish tentacles. The most reproducible results in the analysis

of reducing sugars and sialic acid were obtained after 30-min hydrolysis in 4 N HCl. Maximum yields of hexosamine were obtained after hydrolysis for 1 hr. Internal standards were utilized during all analytical procedures.

The analysis for Mg and Ca was done on an atomic absorption spectrophotometer.¹ Acrylamide-gel electrophoresis² was performed on NS after treatment with several solvents. Aliquots of NS and WT dissolved in methanol were injected into a gas chromatographic apparatus³ in order to quantitate the concentration of cholesterol in these specimens.

Histamine and serotonin assays were performed according to previously described techniques (14, 15). A spectrophotofluorometer⁴ was employed for these experiments. Paper chromatography was used to determine the concentration of quaternary ammonium ions (16). Spectrofluorometric and paper chromatographic techniques were employed to quantitate the catecholamine concentrations (17).

Results. An elemental analysis is shown in Table I. The bulk of this composition is carbon which comprises about one half of NS by weight. Oxygen is the second most prevalent element accounting for one fifth of the mass. Inorganic salts account for less than one eighth of the sample.

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¹ Atomic Absorption Spectrophotometer Perkin-Elmer Corporation, Norwalk, Conn.

² Canal Industrial Corp., Rockville, Md.

³ Packard Gas Chromatograph, Packard Instrument Co., Inc., Downers Grove, Ill. 60515

⁴ Aminco Bowman Spectrophotofluorometer, American Instruments Co., Silver Spring, Md.

TABLE I. Elemental Analysis of Dried Nematocyst Suspensions.

Element	% Dry weight
Carbon	47.95
Hydrogen	7.80
Nitrogen	8.04
Sulfur	1.31
Oxygen	20.59
Sodium	3.12
Chlorine	4.75
Phosphorus	1.35
Magnesium	1.38
Calcium	>0.50

The chemical composition of NS and WT is included in Table II. All the constituents measured here except sialic acid are higher in NS than in WT. Considerable nucleic acids were found but only minimal lipids were detected. The cholesterol determination of a colorimetric analysis were in agreement with results by gas chromatography.

Several protein bands were found after gel electrophoresis of NS treated with 8 M urea. Other solvents such as acids, alkali, alcohol, polysorbate 20 and 2-mercaptoethanol at varying concentrations did not contain material with as many bands.

TABLE II. A Comparison of the Chemical Composition of Nematocyst Suspensions and Whole Tentacles.

Test performed	% Dry weight	
	Nematocyst susp.	Whole tentacles
Reducing sugars	1.87	0.72
Sialic acid	3.76	5.31
Hexosamine	3.74	1.61
Cholesterol	2.09	0.99
Glycerol	0.28	0.13
Saponifiable fats	0	0
Ash	7.74	—
DNA	8.53	2.15
RNA	18.48	6.34
Histamine	5.3×10^{-4}	4.0×10^{-4}
Serotonin	0	—
Catecholamines	0	0
Tetraethylammonium	$<5 \times 10^{-4}$	$<5 \times 10^{-4}$
Tetramethylammonium	$<5 \times 10^{-4}$	$<5 \times 10^{-4}$

Trace amounts of histamine were present in NS. No serotonin could be detected. The chromatographic techniques for demonstrating tetraethylammonium and tetramethylammonium ions, capable of detecting 0.5 mg/g were negative. Catecholamines could not be demonstrated by either chromatographic or fluorometric techniques.

Discussion. The elemental analysis reveals that the bulk of NS is organic (Table I). A nitrogen content of 8% may allow the total of nucleic acid and protein concentrations to approach 50%. This level closely approximates that reported by others (18). 2-Mercaptoethanol was effective in solubilizing some of the NS and also in lysing many nematocysts. These observations suggest that disulfide bonds may account for some of the 1.3% sulfur concentration. The NaCl percentage of dry weight of almost 8% is considerably above that of summer Chesapeake Bay water (approximately 1.5%). The large concentration of oxygen is difficult to account for because of the low carbohydrate and fat content. It is possible that other fats such as terpenes and sterols may be present in significant amounts.

The nucleic acid content of 27% probably indicates that some cellular nuclei contaminated NS. Since the nitrogen content of nucleic acids is almost the same as that of proteins, the maximum protein content of NS then approaches 23%.

The hexosamine and sialic acid percentages of dry weights are similar to that reported by Gross, Dumsha, and Glaser (19).

Fat levels have been reported in *Cyanea capillata* (20) and *Physalia physalis* (21). Middlebrook and Lane reported 9% of the *Physalia pneumatosaccus* was cholesterol (21). Virtually all cholesterol was restricted to this region. *Chrysaora* tentacles thus have the highest reported tentacular cholesterol content. There have been conflicting reports regarding the presence or absence of 5-hydroxytryptamine in coelenterate tissues (22, 23). We were unable to detect its presence in *Chrysaora*.

Several quaternary ammonium bases have been found in coelenterate tissues. In some cases these compounds have been regarded as

toxic agents capable of producing "curare-like" effects on the prey 16). Neither tetraethylammonium nor tetramethylammonium ions were detected in *Chrysaora*.

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