the beetles assembled before the entrance of the *Myrmica* nest (Fig. 1). When odors of various ant species were offered simultaneously, the scent of *Myrmica* was always preferred (Fig. 2). The beetles were even attracted if the *Myrmica* scent was added to an air current carrying the odor of different ant species. Only when *Myrmica* odors were absent did other species of the subfamily Myrmicinae, for example, *Tetramorium caespitum* L. and *Solenopsis fugax* Latr., weakly attract the beetles. Thus, a host-specific odor is the releasing signal which attracts *Atemeles pubicollis*.

Olfactory and anemotactic orientation are intimately connected. An air current without host scent releases only a short turning reaction but no clear upwind crawling. Also, the beetles orient osmoclinotactically, that is, in a zigzag pattern toward the source of the scent. Thus they are able to detect and follow the increasing concentration of the scent in the air current. Together these mechanisms enable the guests to find their hosts with high accuracy. *Atemeles* react to the chemical stimuli coming from *Myrmica* only from 1 to 14 days after leaving the *Formica* colony. After this time, no accumulation of beetles at a *Myrmica* scent source is observed. In laboratory colonies, all beetles are adopted by *Myrmica* soon after they reach the peripheral nest area. Adoption is released by substances which are secreted from integumental glandular cells situated dorsolaterally in the beetles' abdominal segments. These substances are highly attractive for the host ants (*5*).

After hibernating and reaching maturity, *Atemeles* leave *Myrmica* and return to *Formica*. Again the beetles orient to specific odors of their host, which is now *Formica*; the positive anemotactic and osmoclinotactic behavior is identical in beetles migrating to *Myrmica* or to *Formica*.

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Acid Polysaccharides from Invertebrate Connective Tissue: Phylogenetic Aspects

Abstract. *Polyfucose sulfate and a chondroitin sulfate were isolated from echinoderm connective tissue. Coelenterate and poriferan connective tissues were devoid of these acid polysaccharides.*

Mathews *et al.* (*1*) have isolated chondroitin sulfates from the cartilages of a mollusk (squid) and of an arthropod (horseshoe crab). They speculated on the possible existence of a related acid polysaccharide precursor in the connective tissue of some common ancestor. In an investigation on the structure of the carbohydrate moiety of invertebrate collagens (*2*), we have isolated and studied the acid polysaccharides present in the connective tissue of various phyla. We report the isolation of a polyfucose sulfate and a chondroitin sulfate from the connective tissue of an echinoderm (*Thyone briareus*) and the absence of these acid polysaccharides in the connective tissue of a coelenterate (*Metridium dianthus*) and a poriferan (*Hippospongia gossypina*).

Connective tissue from the body wall of *Thyone briareus* was rendered completely soluble with a proteolytic enzyme (Pronase at 70°C, *p*H 8.5 for 2 hours). The acid polysaccharides were precipitated with cetyltrimethylammonium bromide and the precipitate was converted into the soluble potassium salt by potassium thiocyanate and treated with mild alkali (0.1*M* NaOH, 65°C for 2 hours) to remove traces of mannose, xylose, and glucose, as well as residual peptide. The acid polysaccharides, as potassium salts, were separated into two components by the addition of ethanol (Table 1). The optical rotation and composition of the fraction precipitated with 15 to 19 percent ethanol are close to those calculated for chondroitin sulfate, except for excess sulfate. The hexuronic acid moiety was shown to be glucuronic by reduction of the methyl ester to glucose. Hexosamine was identified as galactosamine on an amino acid analyzer. The infrared spectrum in the 8- to 10-μm range indicated that there was a sulfate group in the 6-position of the D-galactosamine residue. The location of the other sulfate groups was not determined. The preparation was completely hydrolyzed (*3*) by testicular hyaluronidase (E.C. 3.2.1.35) (turbidity test and ratio of Elson-Morgan reagent to carbazole). The fraction precipitated by 38 to 52 percent ethanol (Table 1) is a poly-L-fucose sulfate. Preliminary analysis indicated that the L-fucose residues are joined primarily by $\alpha(1\rightarrow2)$ linkages and that the sulfate groups are located at the 3- or 4-position (*4*). Neither of these acid polysaccharides was detected in intact connective tissue, in derived gelatins, or in the carbohydrate fractions of *Metridium dianthus* and *Hippospongia gossypina* obtained by gel filtration after proteolysis. Indeed, no acid polysaccharides (containing hexuronic acid, sulfate, or sialic acid) were found in *M. dianthus* (*5*). Polyfucose sulfate has now been found in the connective tissue of at least three species of echinoderms and in the egg jelly-coat of three additional echinoderm species (*6*). To the best of our knowledge, it has not been reported to occur in any other phylum. It does occur, curiously enough, in one division of plants also consisting of L-fucose residues joined primarily by α-L-$(1\rightarrow2)$ linkages (*7*).

Table 1. Analysis of acid polysaccharides from *Thyone briareus*.

Residues and elements	Molar ratio of ethanol fractions	
	15 to 19 percent (to hexosamine)	38 to 52 percent (to fucose)
Fucose	0.04	1.00
Galactose	0	0.06
Hexosamine	1.00	< .03
Hexuronic acid	1.07	< .02
Sulfate	1.6	.97
Nitrogen	1.02	.04
$[\alpha]_D^{21}$ (water, *c* 1.0)	−42°	−150°

Polyglucose sulfate (*8*) is another example of a connective tissue acid polysaccharide which, thus far, has been encountered in only one animal phylum. The polysaccharide, hyaluronic acid, has been found only in protozoa and in

the higher vertebrates (*9*). The phylogenetic distribution of polyfucose sulfate is somewhat analogous to that of sialic acid. This compound is present in echinoderms, hemichordates, cephalochordates, and vertebrates (*10*), and is produced by the bacterium *Escherichia coli* in the form of a polymer, colominic acid (*11*), but is absent in the intervening phyla (*10*).

Thus, it appears that closely related acid polysaccharides may have had independent biogeneses. Whether chondroitin sulfate is absent in all of the triploblastic coelomates that are possible common ancestors of the mollusks and arthropods and of the echinoderms is not yet known.

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3. The material was hydrolyzed, under the same conditions of temperature and concentration, at a rate approximately 60 percent of that of a sample of chondroitin-6-sulfate isolated from bovine bone.
4. The stability of the sulfate to strong alkali eliminates the possibility of a 1→4 glycosidic linkage [See E. G. V. Percival, *Quart. Rev. Biol.* **3**, 369 (1949)]. The extreme sensitivity of the glycosidic linkage to acid suggests a 1→2 linkage. The L-fucose was isolated and characterized as the 1-methyl-1-phenylhydrazone (m.p. 178.5°) [See E. Percival, in *Methods in Carbohydrate Chemistry*, R. L. Whistler and M. L. Wolfrom, Ed. (Academic Press, New York, 1962), vol. 1, p. 197].
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Thiamine Release from Nerve Membranes by Tetrodotoxin

Abstract. *Tetrodotoxin (3×10^{-8} molar) promoted the release of thiamine from perfused rat and frog nerve preparations in a manner similar to other neuroactive drugs. When the rats were injected with thiamine labeled with sulfur-35, analyses of brain, spinal cord, and sciatic nerve homogenates revealed labeled thiamine in membrane, synaptosomes, and mitochondrial subfractions. However, on incubation of these fractions with tetrodotoxin, thiamine was released only from the membrane fragments.*

Tetrodotoxin, a poison from the Japanese puffer fish, blocks nerve conduction by inhibiting the early inward current of Na^+ (*1*). We present evidence to implicate thiamine as an intermediate in this blocking activity of the poison.

We have previously (*2*) injected ^{35}S-thiamine into either bullfrogs or rats made deficient in thiamine by dietary restriction, subsequently removed either the spinal cord or sciatic nerves, and perfused the preparation with Ringer solution. When a variety of drugs that cause a change in ion movements in nerves was added to the perfusion fluid, radioactive thiamine was released. Thus, acetylcholine ($10^{-6}M$), potassium chloride ($5 \times 10^{-2}M$), ouabain ($10^{-6}M$), and ethylenediaminetetraacetate ($10^{-3}M$) promoted an immediate efflux of the vitamin, whereas choline ($10^{-4}M$) or NaCl ($5 \times 10^{-2}M$) had no effect. However, the most potent agent in releasing thiamine is tetrodotoxin at a concentration of $3 \times 10^{-8}M$ (0.01 μg/ml) (Fig. 1).

We have studied the stoichiometry of this relation with several different concentrations of tetrodotoxin. Scattered results were obtained so that it was impossible to make a definitive statement on the efficacy of the tetrodotoxin-stimulated efflux of thiamine. Nevertheless, while the ratio of the number of moles of tetrodotoxin necessary to release 1 mole of thiamine varied from 2 to 70, with most of the neuroactive drugs that were tested, a ratio of 200 to 400 was observed. The vitamin that was released into the medium (amounting to 0.1 to 1 percent of the total thiamine content of the preparation) was primarily in the form of thiamine monophosphate (TMP) and free thiamine. In the nerve prep-

Table 1. Release of ^{35}S-thiamine from cell subfractions by tetrodotoxin. Fractions were incubated in Krebs-Ringer phosphate buffer, *p*H 7.2, in the presence and absence of tetrodotoxin ($3 \times 10^{-8}M$) for 20 minutes at 37°C. After incubation, samples were centrifuged at 48,000*g* for 60 minutes to deposit the organelle. A portion of the supernatant was then transferred to a vial containing Bray solution, and the radioactivity was determined in a liquid-scintillation spectrometer. Total radioactivity was determined by deproteinization with trichloroacetate.

Cell fraction	Increase over control (%)	Total radioactivity in subfraction (%)
	Brain	
Membrane	65.1	15.6
Synaptosomes	2.3	0.5
Mitochondria	4.2	.9
	Spinal cord	
Membrane	77.3	40.4
Synaptosomes	47.5	10.2
Mitochondria	5.4	1.4
	Sciatic nerve	
Membrane	31.1	13.3
Mitochondria	0	0

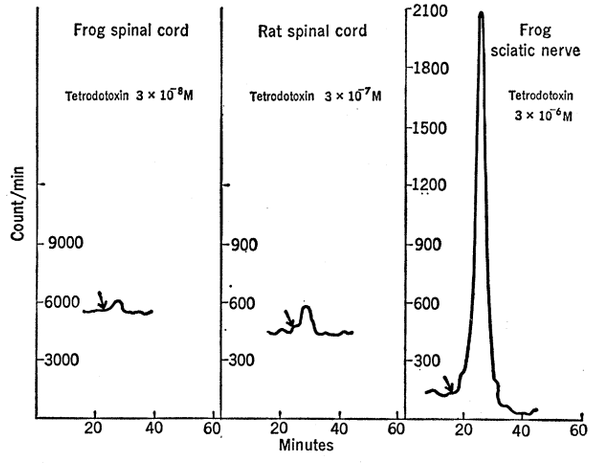

Fig. 1. Effect of tetrodotoxin in promoting the efflux of radioactive thiamine (counts per minute) from perfused preparations. The tetrodotoxin, dissolved in either frog Ringer or Locke solution, was pumped through the nerve chamber at a rate of 1 ml/min for 10 minutes. Parameters of the system were as follows: flow cell volume, 4 ml; rate meter time constant, 40 seconds; chart speed, 7.5 cm/hr. Arrow indicates addition of drug.