employed by others in animal studies [Lucas and Newhouse (*7*), Potts *et al.* (*8*), Freedman and Potts (*9*), Olney (*2*), and Olney and Sharpe (*3*)], who administered MSG in the range of 2 to 10 g per kilogram of body weight. In their latest report, Olney and Sharpe (*4*) reported effects of 1 g/kg, by feeding tube, in newborn mice.

Mayer-Gross and Walker (*10*) demonstrated a transient toxic effect (vomiting) after intravenous administration of 20 g of sodium glutamate to normal adults, while this dose produced a favorable metabolic effect (arousal) in comatose, hypoglycemic patients. To our knowledge, then, no permanent neurotoxic effect for glutamate has been definitely reported for adult humans.

Rapid utilization of MSG would prevent the accumulation of free amino acid in the plasma and would reduce the danger of damage to susceptible tissue. Oral MSG is well utilized by human subjects, as indicated by no significant alterations in concentrations of plasma glutamic acid plus glutamine 12 hours after thrice daily feeding of glutamate formulas. The subjects also were at constant body weight and in nitrogen balance (*11*).

Clearly, glutamate produced metabolic effects consisting of decrease in serum cholesterol, phospholipids, and β-lipoproteins without alterations of body weight, nitrogen balance, liver function, or neurological activity (*12, 13*).

The question of MSG toxicity appears, therefore, to center around the permeability of the blood-brain barrier to glutamic acid. How much of the fed or injected glutamate does actually reach the brain? It is obvious that significant amounts reach the brain in newborn animals and possibly infants. Himwich *et al.* (*14*) have reported ready access of glutamate to the brain at birth but exclusion of it with establishment of a blood-brain barrier by the 10th day. In view of this finding, it is likely that even with parenteral administration of large doses of MSG only small amounts of it would reach the adult brain. In newborn mice and monkeys prior to the establishment of this barrier, the brain could be susceptible to the toxic effects of MSG described by Olney (*2, 3*).

We conclude from our studies that very high oral doses of glutamate (147 g/day) are tolerated, with no neurological changes, by adult gerbils and humans. The problem of determining the age of transition from susceptibility to tolerance for MSG in human subjects remains open.

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Echinoid Skeleton: Absence of a Collagenous Matrix

Abstract. *Lack of hydroxyproline and proline in the calcified distal spines and Aristotle's lantern of the echinoderm* Strongylocentrotus *indicated the absence of a collagenous matrix. The fact that the small amount of collagen present in the base of the spines and in the test with sutures was removed by bacterial collagenase indicates that this collagen was not calcified.*

The structural role of a collagenous matrix in the calcified tissues of vertebrates is clearly established (*1*). However, evidence for the existence of a collagenous matrix in the calcified tissues of invertebrates consists of the amino acid composition and the appearance in the electron microscope (*2*) of the calcareous plates and spines of the sea urchins *Strongylocentrotus droebachiensis* (Müller) and *Lytechinus variegatus* (Lamarck) after treatment with pronase. Two phyla (Porifera and Mollusca) have been shown not to have significant quantities of collagen (*2*). This conclusion was reached because hydroxyproline (an amino acid unique to collagen and elastin) was absent or was a minor component of the amino acids present in the skeletal tissues.

The existence of a collagenous matrix needs to be considered in the light of other types of evidence. During the embryological development of the echinoid skeleton (*3*), a large intracellular crystal of calcite, the initial spicule, is first formed. Subsequent skeletal development occurs extracellularly by crystal growth on the initial spicules. Histological studies (*4, 5*) of the decalcified or untreated skeleton of echinoderms have shown the presence of uncalcified collagenous fibers only at the sutural areas between the calcareous plates and at the basal attachment of the spines to the skeleton. Observations of the intact and fragmented trabeculae from spine or plate with the scanning electron microscope (*5–7*) have led to the suggestion (*6*) that no organic phase was present within the trabeculae. In addition, x-ray diffraction studies (*7, 8*) of the mineral phase indicate that the spines and plates behave as single crystals (*9*) of calcite.

We now demonstrate that collagen or proteins containing proline are a minor component of the skeleton of the echinoid *Strongylocentrotus droebachiensis* and that the collagen that is present represents soft connective tissue.

Dried specimens of *S. droebachiensis* were divided into the distal spine, base of the spine, test with sutures, and Aristotle's lantern (excluding teeth). After the dry weights were obtained, each sample was hydrolyzed by being autoclaved for 5 hours at 120°C in 6*N* HCl. Samples of the hydrolyzate containing 160 to 274 mg of sample were chromatographed (*10*) for hydroxyproline and proline. These imino acids were analyzed by methods previously described (*10*).

The data obtained from the four echinoid tissues are presented in Table 1. An analysis of dog bone, a typical calcified collagen, is included for comparison. Chromatograms of the hydrolyzates showed traces (1 to 8 μg) of hydroxyproline in Aristotle's lantern and distal spine. The small amount (174 to 248 μg) of hydroxyproline present in the test (the "shell") or in the base of the spine suggested that

Table 1. Imino acid composition of echinoid and dog skeletal tissues in the untreated state and after incubation with collagenase from *Clostridium histolyticum*; P, proline; H, hydroxyproline.

Tissue	Untreated			After incubation	
	Hydroxyproline (μg/100 mg)	Proline (μg/100 mg)	Molar ratio (P/H)	Hydroxyproline (μg/100 mg)	Proline (μg/100 mg)
Aristotle's lantern	7.9	51	7.30		
Test with suture	174	223	1.45	0.3	10.
Distal spine	1.3	22	19.1		
Base of spine	248	359	1.64	0.3	5.9
Dog bone	3400	3750	1.25		

collagen was at best a minor component. Similarly, small amounts of hydroxyproline have been found in the test of the sea urchins *Arbacia punctulata* (Gray) and *Echinarachnius parma* (Lamarck) (*11*).

The use of bacterial collagenase on dentin (*12*) or bone (*12, 13*) has shown that in the calcified state these tissues are resistant to the enzyme but that they become susceptible after decalcification at neutral *p*H. These studies suggested that collagenase could be used as a means of distinguishing calcified collagen from collagen of soft connective tissues.

Samples of echinoid tissues known to contain hydroxyproline (test with suture and base of spine; Table 1) were taken from the same specimen of *S. droebachiensis*. The tissues (Table 2) were incubated with purified (*14*) and commercial collagenase (General Biochemicals, Chagrin Falls, Ohio) at 37°C in a shaking incubator. The buffered solution of collagenase was replaced every 2 days for 10 days. Since collagenase appears to be a varying mixture of bacterial enzymes, purified collagenase was used for the first 4 days and commercial collagenase was used for the last 6 days. The enzymatic activity of the collagenases was simultaneously demonstrated on horse Achilles tendon and reconstituted collagen from calf skin. After each 48 hours the buffer solution was decanted off and centrifuged at 30,000*g* for 10 minutes. All supernatant solutions were hydrolyzed with acid and chromatographed for hydroxyproline. The first two solutions were analyzed for proline. After 10 days of incubation, the echinoid tissues were washed three times with distilled water, dried in a vacuum, and weighed. The tissues were hydrolyzed with acid and analyzed for proline and hydroxyproline.

Table 2. Effect of bacterial collagenase on dry weight of echinoid tissues. The incubation mixture contained 5 mg of collagenase per 10 ml of 0.1*M* tris buffer (*p*H 7.5) containing 0.01*M* $CaCl_2$ and Merthiolate diluted 1 : 10,000.

Tissue	Dry weight (mg)	
	Before incubation	After incubation
Test with suture	835	790
Base of spine	855	810

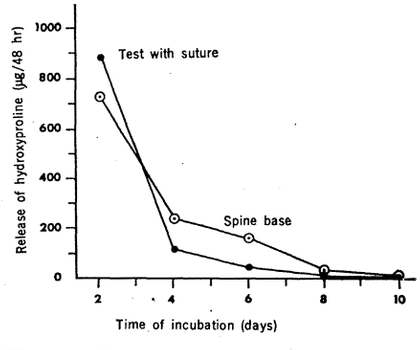

Fig. 1. Effect of bacterial collagenase on the release of collagen (hydroxyproline) from the skeletal tissues of *Strongylocentrotus*. Collagenase liberated 129 and 137 μg of peptide-bound hydroxyproline per 100 mg of test and base of spine, respectively.

Relatively large amounts of peptide-bound hydroxyproline and proline were made soluble by collagenase during the first 4 days of incubation, but by the end of 10 days the amount of hydroxyproline found in the supernatants approached zero (Fig. 1). After the first 2 days of incubation the molar ratios of proline to hydroxyproline in the supernatant were 1.23 and 1.30 for test and base of spine, respectively. These ratios are similar to those found for collagen of mammalian (*1*) or echinoid (*11, 15*) soft tissues. After enzymatic digestion, traces of hydroxyproline and proline were found in the echinoid tissues (Table 1); there was a 5 percent loss in dry weight (Table 2). The trace amount of hydroxyproline represents a maximum of 0.005 percent collagen in the dried samples of test and base of spine. The small amount of proline represents 0.1 to 0.3 percent noncollagenous protein, the significance (*16*) of which remains to be determined.

Since collagenase has no effect upon calcified mammalian tissues in releasing collagen, nitrogen, or calcium (*12, 13*), it is reasonable to assume that the same is true for echinoid skeleton. After enzymatic removal of collagen or proline-containing protein from echinoid tissues, with a minor loss of total dry weight, there is no collagen and little other protein within the skeleton that could act as a matrix. It appears that collagen is not a matrix of the echinoid skeleton. The collagen (*2*) reported to be present in the skeleton of echinoderms is most likely derived from soft connective tissues.

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