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Fossilization of Soft Tissue in the Laboratory

Derek E. G. Briggs and Amanda J. Kear

Some of the most remarkable fossils preserve cellular details of soft tissues. In many of these, the tissues have been replaced by calcium phosphate. This process has been assumed to require elevated concentrations of phosphate in sediment pore waters. In decay experiments modern shrimps became partially mineralized in amorphous calcium phosphate, preserving cellular details of muscle tissue, particularly in a system closed to oxygen. The source for the formation of calcium phosphate was the shrimp itself. Mineralization, which was accompanied by a drop in pH, commenced within 2 weeks and increased in extent for at least 4 to 8 weeks. This mechanism halts the normal loss of detail of soft-tissue morphology before fossilization. Similar closed conditions would prevail where organisms are rapidly overgrown by microbial mats.

Fossils that preserve evidence of soft tissues yield a wealth of information over and above that provided by the normal remains of mineralized shells, bones, and teeth. Structural organic tissues such as cuticle may become incorporated into the fossil record as complex inert biopolymers. Tissues such as muscle, however, which are subject to rapid autolysis and metabolization by bacteria, usually can be preserved only by early authigenic mineralization (*1, 2*). Occasionally such mineralization occurs quickly enough to preserve spectacular three-dimensional detail of muscles and other tissues and thus to give rise to some of the most remarkable fossils known. This type of preservation usually involves calcium phosphate (*3*). Recently studied examples have been found in the Santana Formation (Lower Cretaceous), Chapada do Araripe, Brazil (*4–6*), and the Cordillera de Domeyko (Upper Jurassic) of Chile (*7*). Here the striated muscle of both elasmobranchs and bony fishes displays details of the morphology of the sarcolemma, fibrils, sarcomeres, and the banding within them, and occasionally even rows of cell nuclei (*6, 7*). Mineralization of fish soft tissue is a more widespread phenomenon than is generally realized, but many reports predate the availability of scanning electron microscopy (SEM), and the degree of detail that is preserved is unknown. Phosphatized muscle has also been reported in other groups, including pterosaurs from the Santana Formation (*8*), cephalopods from Solnhofen (Jurassic, Tithonian), Germany (*9*), and Christian Malford (Jurassic, Callovian), England (*10*), and crustaceans from Solnhofen and from within the gut of Santana fishes (*11*).

Attempts to understand the process of soft-tissue mineralization—the so-called "Medusa effect" (*5*)—and the conditions required to promote it have been based mainly on data from fossils. Experiments on mineralization in association with decaying shrimps (*1*) and fish (*12*) have resulted in the formation of calcium soaps, but not in extensive mineralization of soft tissue (*13*). Here we describe how the mineralization of soft tissue can be induced under experimental conditions in the laboratory, and we use the results to interpret its occurrence in the fossil record.

Freshly killed specimens of the shrimp *Crangon crangon* and the prawn *Palaemon* sp. (*14*) were placed in experimental vessels with 50 ml of standard artificial seawater (ASW) inoculated with water (50 ml/liter) and sediment (~0.5 ml/liter) from the Tay Estuary, Dundee, Scotland, and yeast extract (0.1 g/liter) (*15, 16*). The vessels were incubated at 20° ± 0.5°C for 3 days to 25 weeks before sampling (*17*).

We carried out replicate experiments (five separate vessels, one carcass in each, for each sample point) under four different conditions (experiments A to D) to explore the mineralization process. At start-up, the O_2 in the water in experiments A, B, and C was at 50% saturation; in experiment D the water was deoxygenated (*18*). Initial decay reduced the O_2 concentration to just a few percent within 1 to 2 days. Rapid rediffusion of atmospheric O_2 was permitted in experiment A, which was not sealed; in experiment B the jar was fitted with a cap that permitted slow diffusion only; in experiments C and D the systems were sealed with anaerocult, which removes gaseous O_2

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and replaces it with CO_2, in an aluminum bag that prevented reoxygenation (*19, 20*).

Where O_2 was available throughout (experiments A and B), the carcasses displayed four stages of decay. Within 1 week, the abdomen flexed ventrally and the arthrodial membranes stretched as the internal tissues expanded as a result of osmotic pressure. The cuticle began to lose rigidity. The muscles became opaque but retained their internal strength and cohesion. Isolated flat, round, hemispherical or dumbbell-shaped crystal bundles (*21*), up to 0.5 mm in dimension, began to precipitate in the envelopes of cuticle formed by the scaphocerites, limbs, and tail fan. Within 2 to 3 weeks, the carcass split at the junction between the cephalothorax and abdomen. The muscles lost their internal strength; few fibers remained. Crystal bundles began to form in the cephalothorax and tergites. In some experiments they coalesced into a crude pavement on the inner surface of the cuticle. By 4 to 8 weeks (in *Crangon* and *Palaemon*, respectively) the entire cuticle was flaccid, and, although it remained relatively intact, it collapsed on removal from the water. The muscles shrank and in most cases degenerated to an amorphous white semiliquid state; no evidence of fibers remained. By 8 to 20 weeks (in *Crangon* and *Palaemon*, respectively) the legs fell off and the exoskeleton gradually disarticulated; any semiliquid soft tissue that remained was released. Muscle tissue was rarely mineralized in these experiments (none recorded in weeks 4 to 8 in *Palaemon* or *Crangon* under condition B; 13%, $n = 80$ in all *Palaemon* run in experiment B) and consisted only of scraps (<1 mm in dimension).

Where reoxygenation was prevented (experiments C and D), decay followed a similar sequence but some of the muscle tissue usually became mineralized. In addition, the cuticle remained rigid, particularly in the tergites of the abdomen. Crystal bundles sometimes formed. Mineralization of the muscle took place in stages. The muscle developed a pasty texture within 2 weeks, which, if disturbed, collapsed and flowed over the cuticle as in a mud flow. Small scraps of tissue (<1 mm, usually 0.1 to 0.5 mm in dimension) became mineralized within 2 to 4 weeks. In addition to muscle, scraps of the hepatopancreas and complete eggs became mineralized. Within 4 to 8 weeks, blocks of mineralized muscle tissue 1 to 3 mm in size were evident and the hepatopancreas was mineralized in many specimens. The muscle commonly mineralized on the abdominal tergites and sternites, and, as a result, a hollow center to each somite was left where the remainder of the muscle had decayed. Some of the muscle that shrank and collapsed into the lowest part of the space enclosed by the exoskeleton was preserved as additional remnants. Mineralization appeared to be initiated in the hepatopancreas and was most pronounced near the anterior of the trunk; the last two abdominal somites were empty as a result of decay in most cases. In very rare cases the muscle of a limb or chela was mineralized.

The proportion of muscle mineralized and the degree of detail preserved varied both among and within specimens. Mineralization of recognizable muscle tissue in *Crangon* occurred in 53% of the experiments in which the water was oxygenated at the outset (experiment C) and in 60% of the experiments in which the systems were completely anoxic from start to finish (experiment D) (4 to 8 weeks, $n = 15$, in both cases). Mineralization occurred in 67% of *Palaemon* under the conditions in experiment D (4 to 8 weeks, $n = 15$).

The fibers of muscle were mineralized in many experiments, and traces of the banding on the muscle fibrils (probably corresponding to the M lines where the fibrils rupture) were evident in a few (Figs. 1A and 2A). The microspheres of mineral replicating the muscle tissue were normally less than 1 μm in diameter. The sarcolemma was occasionally preserved by a coating of mineral even where the fibers it enclosed had decayed (Figs. 1B and 2B). In a few experiments just an outline of the sarcolemma remained as a series of polygons on the inner surface of the tergites with some trace of the ends of the fibers within (Fig. 2C). Eggs, which are ~0.5 mm in dimension, were normally covered with an irregular, relatively smooth membrane. Inside this, scattered irregular spheres up to 5 μm in diameter occurred within a disorganized open meshwork (Fig. 2E). Bacteria were rarely mineralized (Fig. 2F).

The composition of the crystal bundles differs from that of the mineralized soft tissues (Table 1). Analysis with an electron microprobe revealed that the crystal bundles are essentially pure calcium carbonate (x-ray diffraction indicates a mixture of aragonite and calcite). The mineralized

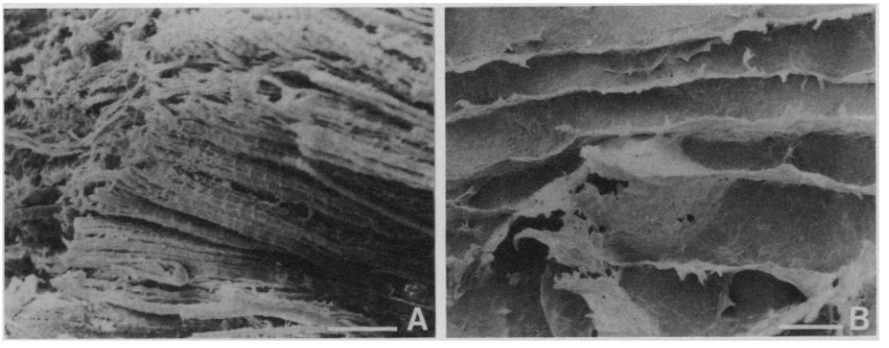

Fig. 1. (**A**) Fresh muscle fiber from *Palaemon* sp. Z bands are evident on the individual fibrils. Scale bar, 10 μm. (**B**) Sarcolemma in a torn *Crangon crangon* muscle that decayed for 1 day with rapid diffusion. Scale bar, 20 μm. Both specimens were fixed in 1% glutaraldehyde in 0.1 M cacodylate buffer and prepared for SEM by the hexamethyldisilazane method (*31*).

Table 1. Average composition of mineralized tissues in *Crangon* and *Palaemon* (*P*) at specified decay times. Oxide weights are based on electron microprobe analyses (total given as the percent by weight of sample mineralized). The ratio of calcium phosphate to $CaCO_3$ is based on the assumption that all P_2O_5 is incorporated into ideal OH-apatite [$Ca_5(PO_4)_3OH$]. The CaO:P_2O_5 ratio is 1:1.32 (based on molecular weights, ignoring hydrogen and excess oxygen). The remaining CaO is assumed to form $CaCO_3$.

Mineralized tissue	Sample mineralized (% by weight)	Na_2O	MgO	SiO_2	P_2O_5	SO_3	CaO	CaO in amorphous phosphate	Ratio of calcium phosphate to $CaCO_3$ (%)
Crystal bundle, 8 weeks	51.54	0.8	1.6	0.01	1.2	0.6	47.3	1.6	3.4:96.6
Muscle, 8 weeks	40.18	1.0	1.2	0.03	14.9	0.6	22.5	19.7	87.6:12.4
Fresh cuticle	44.22	1.3	0.8	0.13	2.1	0.4	39.3	2.8	7.1:92.9
Cuticle, 15 to 25 weeks (*P*)	55.89	0.3	1.7	0.17	19.0	1.4	32.7	25.1	76.8:23.2
Egg, 25 weeks (*P*)	16.85	0	0.3	0.11	3.5	0.4	11.8	4.6	39.0:61.0

muscle tissue, on the other hand, is predominantly calcium phosphate (>80%). The lack of definite peaks in the x-ray diffraction pattern indicates that this material is amorphous; calcium soaps may also be present. The amount of phosphate in the cuticle also increased with decay. The eggs were less completely mineralized than the other tissues with a much lower proportion of phosphate to carbonate.

The formation of calcium phosphate in sediments is induced by microbial action (*22*). Recent discussion of the circumstances surrounding the phosphatization of soft tissue has focused mainly on the fossil fishes of the Cretaceous Santana Formation of Brazil (*4, 7, 23*). There are striking similarities between the features of our experiments and those of the Santana fishes. In both, preservation is variable both among and within specimens (*23*), the muscle tissue is only partially mineralized and in many cases it has collapsed within the body cavity (*4, 23*), similar ultrastructural details of the muscle tissue are retained, and the mineral textures are similar (*11, 23*). Muscle tissue of fishes from the Santana Formation is preserved in calcium hydroxyapatite and calcium fluorapatite (*11*). The conditions responsible for the preservation seen in the Santana Formation, however, including the mineral composition [it has been argued that the bulk of muscle tissue is preserved in calcite (*7, 24*)], the pH and O_2 concentration of the seawater, the source of ions, and the rate of mineralization, remain controversial.

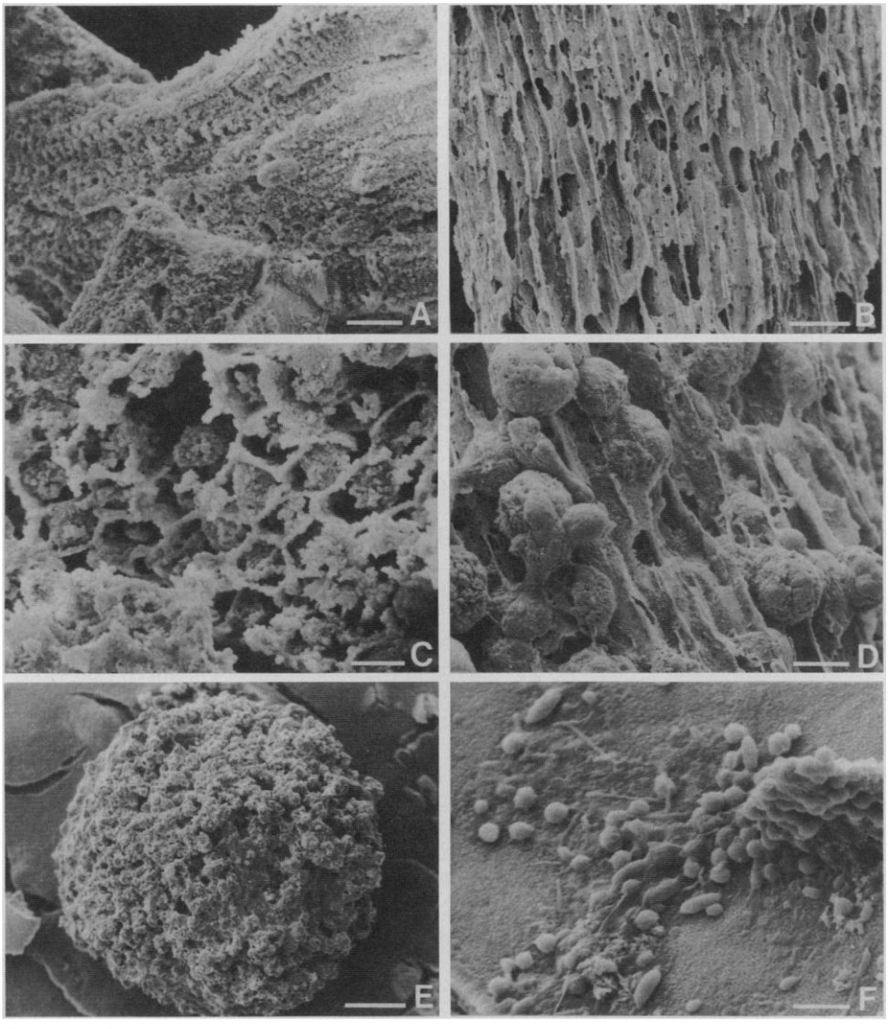

Fig. 2. (**A**) Banding (probably M lines) on mineralized muscle fibrils from the sternite of *Palaemon* after 15 weeks under the conditions of experiment C. Scale bar, 20 μm. (**B**) Mineralized sarcolemma from the same specimen in a fragment where the fibers have decayed away. Scale bar, 100 μm. (**C**) Polygonal pattern of mineralized sarcolemma on the tergite cuticle of *Palaemon* after 10 weeks under the conditions of experiment D. A clump of mineralized fiber ends is attached within each polygon. Scale bar, 10 μm. (**D**) Dumbbell-shaped crystals overgrowing mineralized sarcolemma [same specimen as in (A) and (B)]. Scale bar, 40 μm. (**E**) Partially mineralized egg of *Palaemon* with the outer membrane decayed away after 20 weeks under the conditions of experiment D. Scale bar, 100 μm. (**F**) Mineralized bacteria on the limb cuticle of *Palaemon* after 15 weeks under the conditions of experiment D. Scale bar, 4 μm.

Even where O_2 was present at the outset (experiments A through C), it was eliminated in 1 to 2 days with decay of the carcass. However, substantial phosphatization of muscle tissue only occurred where the system was closed (experiments C and D) in an aluminum bag. In the case of the Santana fossils a prokaryotic mat may have played a similar role (*4*). In closed conditions pH fell rapidly from 8 to between 6 and 7 at 3 days; it then recovered to ~8 within 4 weeks. This reduction in pH is a result of anaerobic decay and the buildup of CO_2 and volatile fatty acids, which cannot escape. It may have been slightly enhanced by the CO_2 produced by the anaerocult (*25*), but the reduction was less than the drop (to pH 6) produced by the decay of larger worm carcasses in conditions of slow diffusion (experiment B), that is, without anaerocult (*16*).

Lowering the pH generally increases the stability of apatite relative to $CaCO_3$ (*26*). It has been argued that a drop in pH in the vicinity of a decaying Santana fish carcass was necessary to inhibit carbonate precipitation and to promote the formation of apatite to fossilize the soft tissue (*4, 27*). Under open conditions (experiments A and B), on the other hand, pH remained just below 8 at week 1 but then rose to about 8.5 and remained at that level beyond week 8. Soft tissue was rarely preserved, and crystal bundles formed. In some closed experiments dumbbell-shaped crystal bundles 100 to 200 μm long precipitated over mineralized muscle tissue (Fig. 2D). Electron microprobe analyses confirmed that the crystal bundles are composed of $CaCO_3$, whereas the muscle is calcium phosphate (Table 1). We have observed this overgrowth relation only in specimens that have decayed for 8 weeks or more, by which time pH values had recovered to ~8.

Some models for the fossilization of soft tissue in phosphate have involved the buildup of phosphate to critical concentrations in sediment pore waters as a result of microbial decomposition of organic material (*4, 10*); other models have suggested that the organism itself is the primary source of phosphate (*9, 28*). Our results confirm that some soft tissue can be partially phosphatized even where the sole source of phosphate is the organism itself (3.0% dry weight in *Crangon* carcasses, 2.05% in *Palaemon*, no phosphate in ASW). Additional phosphate would presumably be necessary to mineralize large carcasses extensively, but the preferential fossilization of soft tissue in phosphate in

particular groups, such as fishes, arthropods, and squid, is presumably a reflection of their composition. No mineralization occurred in the polychaete *Nereis* (1.48% phosphate by weight) under the same experimental conditions.

Phosphatization of Santana Formation fishes has been estimated to occur in life (*7*) or, on the basis of a comparison with decay rates of modern fish tissues, within 5 hours of death (*29*). In our experiments, however, the process was initiated within 2 weeks, but the degree of mineralization of muscle tissue increased up to 4 weeks or even longer. The rate of decay, and presumably mineralization, would have been further slowed at lower temperatures.

This study, together with the evidence of the fossil record, emphasizes that precise conditions must prevail in order for mineralization of soft tissue to take place. But, once the morphology is stabilized by initial mineralization, the potential for preservation of the soft tissue through subsequent diagenesis is enormously enhanced, although the material remains vulnerable to compaction unless subsequently enclosed in a concretion. There is a critical balance between decay to release phosphate and precipitation to preserve the soft tissue before much morphological detail is lost (*1*, *7*). This balance occurs in closed conditions where an initial drop in pH triggers phosphatization. The role of microbial films in fossilization may extend beyond the protection of carcasses and inhibition of decomposition (*30*) to the establishment of the conditions necessary for rapid mineralization (*4*).

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Population Structure and the Evolution of Virulence in Nematode Parasites of Fig Wasps

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It is often assumed that parasitic and disease-producing organisms tend to evolve benign relationships with their hosts over time. In contrast, theoretical arguments suggest that increased opportunities for parasite transmission will promote the evolution of increased virulence. The natural history of species-specific nematodes that parasitize fig-pollinating wasps permits the testing of these predictions in natural populations. For 11 species of Panamanian fig wasps, those species characterized by population structures that result in increased opportunities for parasite transmission harbor more virulent species of nematodes. In addition, differences in population structure are also associated with differences in other intra- and interspecific phenomena, including sex ratios among the fig wasp species, the degree of tension in the wasp-fig mutualism, and lethal combat among the males of parasitic wasps.

Parasitic and disease-producing organisms influence nearly all aspects of biological organization. These organisms have been implicated either directly or indirectly in (i) limiting the geographical range of host organisms (community composition); (ii) regulating host population densities and dynamics; (iii) mediating the outcome of competition or predation between their hosts and other organisms; (iv) maintaining polymorphisms in blood proteins, and possibly in maintaining general genetic polymorphisms; (v) underlying sexual selection; and (vi) serving as a selective force leading to the predominance of sexual reproduction (*1*–*6*). The proposed mechanisms by which

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