quired a relatively higher *p*H and longer periods of exposure to the reducing agents (*14*). Regardless of the reagent used, it was not possible to dissolve the uneverted threads nor the barbs of the everted ones, even at a *p*H as high as 11. These observations show that the disulfides of the thread are not available for reduction until the thread everts. Also, although these observations cannot account for the insolubility of the barbs, they do suggest a means for purifying the barbs for future analyses.

Disulfide reducing agents are not known to dissolve other proteins containing hydroxyproline. Accordingly, we obtained negative results with collagen of bovine tendon and with mesoglea from *Aequorea aequorea*. Hydroxyproline is present in significant amounts in mesoglea (3.6 percent) of scyphomedusae (*16*) and in the mesoglea (2 to 3 percent) of the hydromedusae used in these experiments. On the other hand, we find nematocysts from hydra and *Physalia*, both rich in hydroxyproline (*4–6*), are dissolved in alkaline thioglycolate.

The nematocysts were treated with performic acid, a reagent which converts disulfides to sulfonates. After the acid was added to them, nematocyst capsules buckled in a manner similar to that observed when they were treated with reducing agents. In performic acid, however, dissolution did not follow. The nematocysts treated with performate stained with alcian blue; this staining procedure is used to detect proteins containing disulfides, such as keratins (*17*). Such staining of the capsule wall and thread did not occur unless the treatment with performate preceded the application of the dye. Furthermore, neither buckling nor dissolution occurred in sodium citrate, formic acid, alkaline sodium glycolate, methionine, or ethanol.

To determine the number and relative sizes of the subunits released from the nematocyst by reduction with disulfide, electrophoresis of the dissolution products on acrylamide gel was analyzed. Samples of dried, discharged nematocysts (0.5 mg) were dissolved either in 0.1 ml of 0.25*M* thioglycolate, *p*H 9.6, or in 6.5 $\times$ $10^{-3}M$ dithiothreitol, *p*H 9.6. The samples were applied to tubes containing 7.5 percent gel in a tris(hydroxymethyl) aminomethane-glycine buffer, *p*H 8.5, and the current adjusted to 5 ma per tube of gel. Gels were stained in amido-schwarz or Coomassie blue. Those stained with amido-schwarz were destained electrophoretically in 7.5 percent acetic acid. As little as 0.1 μg of protein (Armour serum albumin) could be detected with these dyes.

Electrophoresis of dissolved nematocysts repeatedly resulted in one sharp detectable band. Since the migration of this band was approximately 80 percent of the distance traveled by the buffer front, as compared with 48 percent for serum albumin, the protein may be of low molecular weight, or of high negative charge, or both.

Our results resolve the apparently contradictory findings that nematocysts show some chemical characteristics of both collagens and keratins. Furthermore, they help explain such properties of nematocysts as their resistance to gelatinization by autoclaving (*5*), a property usually associated with collagens rich in hydroxyproline. Perhaps the disulfide bridges help stabilize the protein of the nematocyst so that gelatinization does not occur. The resistance of nematocysts to digestion by collagenase might be similarly explained. The differential solubilization of the capsule and everted thread (*14*) may reflect variations either in the aggregation of the nematocyst protein or in the association of that protein with various polysaccharides.

As pointed out by McBride and Harrington (*7*), some of the previously described collagens (or collagen-like proteins) may also have cystine cross-linkages. Piez and Gross (*18*) report the presence of cystine in some collagens isolated from sponges, coelenterates, and echinoderms. Hence, it might be profitable to reexamine other collagens.

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14. The times for the capsule to dissolve at the respective stated *p*H values: (i) in 0.0065*M* dithiothreitol at *p*H 9.4, 8.5, and 7.5 were 1, 3, and 9 minutes; (ii) in 0.25*M* sodium thioglycolate at *p*H 9.6, 8.9, 8.1, 7.1 and 6.2 were 1, 3, 29, 90, and ∞ minutes; (iii) in cysteine at *p*H 10.1, 9.1, and 8.6 were 1, 3, and ∞ minutes; and (iv) in 14.2*M* mercaptoethanol at *p*H 8.4 were 5 minutes. The infinity symbol designates that no visible dissolution took place during 2 hours of observation. The everted threads, although observed under all the above conditions, dissolved only in the dithiothreitol at *p*H 9.4 and 8.5 in 3 and 30 minutes, respectively, and in sodium thioglycolate at *p*H 9.6 in 5 minutes.
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Pholidostrophiid Brachiopods: Origin of the Nacreous Luster

Abstract. *The "nacreous" luster characteristic of the pholidostrophiid group of fossil brachiopods results from a shell structure that produces superimposed sets of natural optical-diffraction gratings made of calcite. The wall structure is crossed lamellar, parallel to the shell surface; thus flakiness and development of reflecting surfaces are facilitated.*

The iridescent pearly luster characteristic of many molluscan shells is commonly found in the shell layer that is composed of horizontal lamellae consisting of many tabular aragonite crystals, and which is referred to as the nacre or nacreous layer. Shell layers that are composed of similar lamellae but mineralogically made up of calcite crystals are referred to as calcitostracum (*1*). The luster of calcitostracum is seldom as well developed as it is in most nacreous layers; thus it is species whose shells are wholly or partly aragonitic that are of commercial interest as sources of pearls (cultured and natural) and mother-of-pearl.

The luster associated with this nacre results from two combined optical phenomena (*2*): (i) thin-film interference

and (ii) diffraction. First, the interference colors result from the reflection of light from the horizontal lamellae of tabular aragonite platelets of varying thickness—usually less than 1 μ and averaging near 0.6 μ. Second, light is diffracted by the irregular edges of the imbricated spiraled or parallel crystal lamellae. The luster seen in the calcitostracum is produced by similar effects, but is usually less well developed because of the fewer edges available for diffraction. Wada (*3*) states: " . . . the relation between the spiral growth and the kind of mollusks indicates that spiral growth is absent from the inner surface of shells consisting of calcite and in aragonite shells without any pearl luster."

The iridescent pearly luster of the Paleozoic pholidostrophiid brachiopods (*4*) has falsely led many investigators to suspect the presence of aragonite, which is common in well-preserved fossil cephalopods. Williams (*5*) observed that " . . . the rather loose foliation of the [calcitic] platelets explains why the pholidostrophiid shells flake so easily and why they have a sheen something like nacre"; because the mineralogy is calcitic rather than aragonitic, he suggested adoption of the term "pseudonacreous."

While the loose foliation of calcite platelets certainly contributes to the pearly luster by means of thin-film interference, the iridescence seen under a stereo microscope is unusual for calcitic shells. It is interesting that commonly not all individuals of a pholidostrophiid species from one locality exhibit a nacreous luster; a population often shows intergradation between dull shells and those with a nacreous luster.

Two common Devonian representatives were selected for study: (i) *Pholidostrophia nacrea* (Hall) from Thedford, Ontario, and (ii) *P. gracilis gracilis* (Imbrie) from Alpena County, Michigan. The shells were ultrasonically cleaned in distilled water and dried. Fresh surfaces were obtained by carefully flaking-off small pieces of the shell with a needle. The pieces were then replicated with carbon *in vacuo* for study and photography under the electron microscope.

The corrugated appearance of a freshly fractured surface from *P. gracilis gracilis* (Fig. 1) is produced by the regular arrangement of needle-like calcite crystals. Different groups of crystals have different orientations. The degree of corrugation is quite variable,

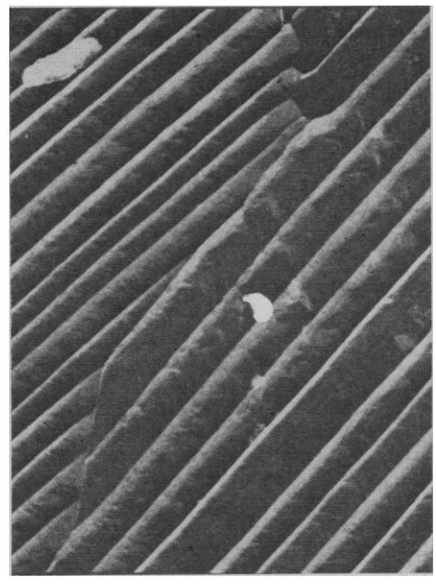

Fig. 1. Carbon replica of a freshly fractured surface (about 6 μ wide) of *Pholidostrophia gracilis gracilis* (Imbrie), showing the natural optical-diffraction grating consisting of calcite.

ranging from heavily impressed (Fig. 1) to almost perfectly flat, even within the same specimen. The orientation of the crystals in one layer is crossed lamellar with respect to crystals in the layers above and below and within the same layer; as a result the angles between groups of crystals can vary from a few degrees to almost 90 deg. The crystal lamellae often indent one another, producing the most-commonly observed crisscross pattern of *P. nacrea* (see cover). The crossed-lamellar structure described by Bøggild (*6*), which is characteristic of and restricted to certain groups of mollusks, differs from that shown in our figures in that the former is usually larger in size and is made up of lamellae crossed perpendicular to the shell surface rather than parallel with it.

Thus the origin of the pearly luster in the pholidostrophiid brachiopods becomes clear when one considers the shell surface, over a limited area, as an optical-diffraction grating with ruling that varies from 10,000 to 25,000 lines per centimeter. Furthermore, a small area of the shell of limited thickness may be regarded as a sequence of superimpositions of diffraction gratings of different orientations. Apart from the birefringence of calcite, the spacing between crystals on the surface layers and the angle of incidence of the light control the wavelength, and thus the color, of the light reflected from the surface, in accordance with the well-known Bragg equation. It is clear from the variation in both crystal spacing and orientation in different parts of a pholidostrophiid shell that a multitude of colors could be produced in satisfying the conditions of the Bragg equation for a given angle of incidence; in this respect some precious opals (*7*) are similar. In addition, the variation in degree of corrugation between one portion of a shell and another produces a surface luster ranging from very highly reflecting to rather dull, and this effect, in turn, exerts control over the intensity of the diffracted light. Irregular crystal spacing and lack of this variation in corrugation would tend to produce a dull, milky appearance—such as on the weathered surfaces of many pholidostrophiid brachiopods. This type of structure probably also occurs in a population of unweathered individuals that exhibits a dull luster throughout.

A large portion of the light incident on the surface is reflected from the surface in the manner described. However, second-order effects result when light passes through more than one of the layers that make up the surface, is reflected by an internal layer, and then returns through the overlying surface layers to a point of observation. This phenomenon is optically similar to the passage of light through a series of two or more diffraction gratings of different orientation before its reflectance back through the diffraction gratings to a point of observation. These second-order phenomena, together with those caused by the extreme birefringence of calcite, doubtless result in complex destructive interference effects. That all of these effects are operable is demonstrated by the fact that the colors are best seen on the carbon replicas, which are of necessity a representation only of the surface features; this we consider good evidence that the diffraction-grating phenomenon is of primary importance.

The unusual occurrence of a patchwork, crossed-lamellar structure made up of layers of more or less regularly spaced calcite needles, that result in the formation of natural optical-diffraction gratings, explains the pseudonacreous luster observed in the Paleozoic pholidostrophiid brachiopods.

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Sterilization by Electrohydraulic Treatment

Abstract. *Electrohydraulic treatment was applied to suspensions of* Escherichia coli, *spores of* Bacillus subtilis *var.* niger, Saccharomyces cerevisiae, *and bacteriophage T-2, as well as to raw municipal sewage. These suspensions were all sterilized. Data are presented to show the different degrees of treatment required for each microorganism.*

Electrohydraulics is a new process for converting electrical energy directly to other useful forms of energy. It depends upon the discharge of a high-voltage arc under the surface of a liquid medium. Arc discharge results in the generation of extremely rapid-rise, high-pressure shock waves and highly active chemical species. The process results in no perceptible rise in temperature; it is virtually instantaneous and completely electrical in nature. Except for a trace of electrode-erosion products formed by the arc, no foreign materials or chemicals are added.

Suspensions of various microorganisms were subjected to multiple electro hydraulic discharges which resulted in complete sterilization of the medium, but no morphologic alteration in the microorganisms studied was observed after this treatment. The total energy necessary for complete sterilization varied with the species.

The electrical equipment consists of a power source fed to a high-voltage d-c power supply, a capacitor bank, charging resistor, and the necessary triggering switch circuit. Energy is stored in the capacitor and discharged to an underwater arc gap. In operation the capacitor is charged to the desired voltage by the power supply, thus storing energy: $J = \frac{1}{2}CE^2$, where J is the energy in joules or watt seconds, C is the capacitance in farads, and E is the voltage. The electrohydraulic effect is obtained by dumping (discharging) this stored energy through a cable into the gap located in the sterilizing tank containing the liquid to be treated.

The equipment used was capable of operating at variable voltage, to 14 kv, at a capacitance from 5 to 95 μfarad, and had an inductance in the range of 2.5 μhenry. It was designed to operate automatically at an arc discharge rate of 0.8 to 30 times per second. The repetition rate, however, had to be chosen commensurate with the energy discharged, so that the power-supply charging rate was not exceeded. The sterilizing tank consisted of a 5-inch (12.7-cm) length of schedule 160 stainless steel pipe, 5 inches in diameter, with flanges welded at both ends to accommodate bolted lids sealed with O-rings (Fig. 1). Electrodes covering a range of gap sizes were fabricated.

Cultures of the following organisms were tested: *Escherichia coli* ATCC 11229, *Bacillus subtilis* var. *niger* ATCC 9372, *Saccharomyces cerevisiae* ATCC 2338, and *E. coli* T-2 bacteriophage. Raw sewage was also tested in an effort to demonstrate efficiency in treating material composed of a naturally occurring variety of microorganisms.

Table 1. Electrohydraulic energy required to kill bacterial and viral species suspended in distilled water. The total time of treatment was less than 1 minute.

Organism	Initial conc. (cells or particles per milliliter)	Total energy (watt hr/gal)
Escherichia coli	2.2×10^7	6.9
Spores of *Bacillus subtilis* var. *niger*	9×10^5	10
Bacteriophage T2	10^8	10
Saccharomyces cerevisiae	9.3×10^6	31
Raw sewage	1.7×10^4	5

Escherichia coli was cultured on nutrient agar slants in milk dilution bottles, at 37°C for 18 to 20 hours. The cells were suspended by gentle washing in 0.01*M* phosphate buffer and were passed through Whatman No. 1 filter paper. Immediately prior to treatment they were diluted in a 10- to 100-fold volume of sterile distilled water. The number of viable cells was determined by duplicate plate counts in nutrient agar, the average number being 10^8 per milliliter. *Bacillus subtilis* var. *niger* was cultured in similar manner, at 37°C for 48 hours. After the cells had been washed from the agar surface with buffer solution and filtered through filter paper, the suspension was treated at 56°C for 30 minutes to destroy any vegetative cells, leaving only spores.

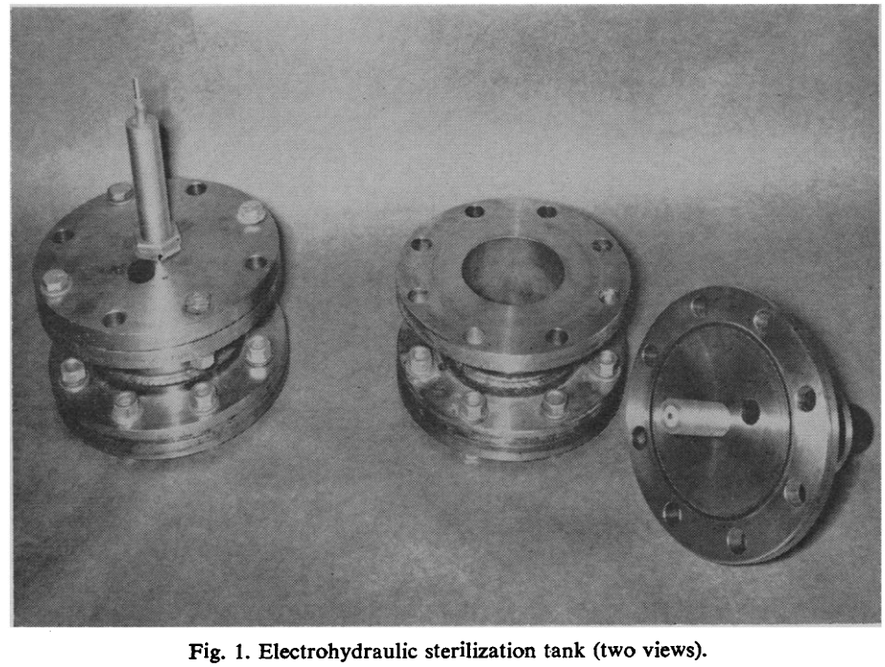

Fig. 1. Electrohydraulic sterilization tank (two views).