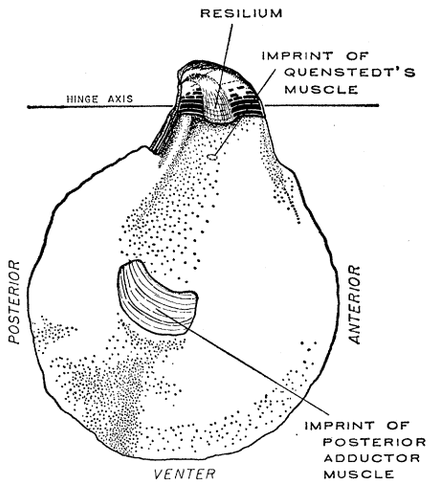

Fig. 1. The left valve of *Crassostrea virginica* (about × 0.6).

the best, although the muscles have been seen or commented on, or investigated by, several other authors (*5*, *6*), some of whom were unaware of the earlier work and claimed to have discovered them. I have found the places of insertion in all fossil and living species and genera available to me, provided the valves were well enough preserved. The conclusion is justified that these muscles, for which the name Quenstedt's muscles is proposed here, are present in all the Ostreidae.

In an average full-grown oyster, the muscles are 1 to 3 mm in diameter at their insertion on the valves, and the places of insertion are 3 to 13 mm from the ventral border of the ligament. The pads on which they are inserted are orbicular, or oval to elongate, and consist of aragonite as can be demonstrated by staining with Feigl's solution.

Each muscle rises from its valve at an angle of about 50 degrees and thins as it extends inward obliquely, in a slightly ventral direction, to pass close by the groove that separates the mantle fold from the outer labial palp. Then it turns to the anterior, splits, and spreads out. Most of its fibrils end in connective tissue between the inner and the outer lamella of the outer demibranch, at the dorsal, or adoral, end of this demibranch. Although the two opposite muscles converge and come close to meeting at the midline, they do not merge, as has been demonstrated by Herdman and Boyce (*4*) and confirmed by Elsey (*6*).

These muscles are very probably modified pedal muscles. However, adult oysters have no traces of a foot left and the muscles must serve other functions. No direct observations concerning their function have ever been made, but from their arrangement it has been surmised that they draw the labial palps and the dorsal, or adoral, ends of the gills forward and outward (*4*) or adjust the position of the ends of the gills (*5*). In view of the uncertainty concerning their present function and concerning their exact homology with one of the several pairs of pedal muscles in normal Bivalvia, it is best to call them Quenstedt's muscles.

As long as the size of the oyster increases by growth, new pads must be laid down, because the muscles increase in size and shift their position toward the venter. New pads are laid down over old ones, but extend beyond them on the ventral side. In this fashion there develops a continuous stack of such pads, ending in the last pad occupied by the muscle. These stacks, consisting of aragonite, are buried within the calcitic shell material of the valves so that only the last pad remains visible. The stack belonging to the adductor muscle is known as the hypostracum. The one belonging to Quenstedt's muscle is best called a myostracum, a term introduced by Oberling (*7*) for all muscle pads and their stacks.

The results of investigations presented here are based on the adult shells of the common oyster species *Crassostrea virginica* (Gmelin, 1791), living today on the Gulf and Atlantic shores of North America. Although this species furnished most of the material investigated, countless other species and genera of the family, living and extinct, have been studied. The results given here have been found applicable in every case studied; it is likely that they apply to all adult oysters from the Late Triassic to today, although no Jurassic or Triassic oysters were found well enough preserved for testing.

It is not the intention here to claim priority of discovery of the aragonitic composition of the adult oyster hypostracum. Oberling (*8*), in his dissertation, dated 7 March 1955, was well aware of this fact. Also, he quoted R. W. Graves as stating in a letter dated 8 June 1953, that K. E. Chave had analyzed one sample of *Crassostrea virginica* and had found its hypostracum to consist entirely of aragonite. Before I began testing for aragonite, my colleague Otto Majewske called my attention to Oberling's work and to the aragonitic composition of the oyster hypostracum, which he had already tested. These observations were pointed out to P. S. Galtsoff in a letter I wrote on 18 August 1961. Very recently, Heinz A. Lowenstam wrote me that he had used x-rays to prove the aragonitic composition of the hypostracum, because he had been led to expect to find aragonite there when he read the article on aragonite in the resilium of oysters (*1*).

H. B. STENZEL
Shell Development Company, Exploration and Production Research Division, Houston, Texas

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17 July 1963

Ammonia: Possible Use for Preserving Fish

Abstract. *Eviscerated oil sardines* (Sardinella longiceps) *that have been treated with ammonia can be stored at a temperature of 25° to 30°C for more than 2 months without deterioration of their nutritive value. There is no measurable residue of ammonia in the final product.*

In many parts of the world, large catches of good edible fish become available with short seasons. When facilities for cold storage and refrigerated transport are inadequate, major parts of the catches, especially in countries like India, are not used effectively. Spoilage is extensive, and the fishermen and traders get compartively poor returns. In such a fish as the oil sardine (*Sardinella longiceps*), the crudely prepared oil becomes the main product, while the more valuable body tissue, which contains proteins of high quality, is dried in the sun to make products which are not suitable for human consumption.

In the tropics, the spoilage of the fish starts within a few hours after the catch. If these changes could be prevented, the fish could be stored in bulk

Table 1. Ammonia content of oil sardine preserved with ammonia, of oil sardine preserved with ammonia and washed repeatedly with water, and of fish flour prepared from preserved, washed fish.

Moisture (%)	Ammonia (mg/100 g)	*p*H
Fish preserved 2 months		
76	458	9–10
Fish preserved 2 months and then washed		
76	55	7.5–8.0
Fish flour made from preserved, washed fish		
3	Trace	6.9

at ordinary temperatures for later processing into fish flour.

We have explored the possibility of treating fish with a safe and easily obtainable agent that has a good preservative action and can, at the same time, be removed easily during drying or other processing. Ammonia in liquid or gaseous form has proved to be promising. Our experience has so far been confined only to a few varieties of marine and fresh-water fish, such as, oil sardine, "Bombil" (*Harpodon nehereus*), *Barbus carnaticus,* and *Labeo* sp. We immersed the eviscerated fish (10 kg) in an equal volume of 1*N* ammonia solution for about 1 to 2 hours and then transferred it to an air-tight vessel (capacity, 20 kg) fitted with a perforated disk about 5 cm above the bottom. In the space below the perforation was some ammonia in solution (2*N*), or paper pulp or another medium soaked in ammonia. The device maintains a concentration of ammonia vapor in the space but does not add appreciably to the moisture content of the fish. The procedure helps to minimize the dissolution of proteins by the ammonia. The fish we have preserved have a *p*H of about 10, and have already kept for over 2 months at a temperature of 25° to 30°C. The excellent condition of the fish suggests that a much longer storage life may be expected.

The treatment is comparatively easy. We have found (Table 1) that, after drying and processing (ethanol extraction for fish flour production), there is no measurable residue of ammonia in the final product as estimated by the Conway diffusion method (*1*). The preserved fish is free from pathogens and has a low bacterial count.

Most of the ammonia from treated fish was leached into the medium by repeated washings with potable water or seawater. Treated fish became soft and continued to remain soft even after repeated washings. Treatment of the washed fish with 0.1*N* citric acid or 0.2-percent calcium chloride improved its texture and made it even firmer than the freshly caught fish.

Further studies have shown that the available lysine, methionine, cystine, and tryptophan and the protein efficiency ratio of fish flour prepared from the preserved fish are not appreciably different from those of flour prepared from fresh, untreated fish (Table 2).

The condition of oil sardines is not changed by the treatment, but "Bombil," which has a much higher water content (90 percent) has a tendency to drip. This does not, however, affect the keeping quality.

Comparative studies with other methods of preservation have shown that ammonia treatment has several advantages over the others. Strong bases (*2*) such as caustic alkali (*3*) and various chemical preservatives (*4*) tend to affect the quality or to leave residues.

V. SUBRAHMANYAN, N. L. LAHIRY
M. N. MOORJANI
R. BALAKRISHNAN NAIR
M. A. KRISHNASWAMY
Central Food Technological Research Institute, Mysore 2, India

Table 2. Protein efficiency ratios (at 4 weeks) and available lysine, methionine, cystine, and tryptophan (in grams per 100 g of protein) in fish flour prepared from fresh oil sardines and from ammonia-preserved oil sardines.

Protein efficiency ratio	Lysine	Methionine	Cystine	Tryptophan
Flour from untreated fish				
3.5	8.96	4.0	1.2	0.94
Flour from treated fish				
3.2	8.74	3.7	1.2	0.94

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31 July 1963

Cytochromes of a Blue-Green Alga: Extraction of a c-Type with a Strongly Negative Redox Potential

Abstract. *Aqueous extraction of lyophilized* Anacystis nidulans *cells followed by chromatography on diethylaminoethyl cellulose separates three different c-type cytochromes. Of the two present in highest concentration, cytochrome-554 has a +0.35-volt redox potential and resembles the cytochrome* f *of other photosynthetic tissues, while cytochrome-549 has a −0.26-volt potential. The possible participation of cytochrome-549 in electron transport in photosynthesis in this alga at a more negative oxidation-reduction potential than previously postulated for any cytochrome is inferred from the similarities in its spectra to the light-induced spectral changes in vivo observed by others.*

The participation of cytochromes in photosynthesis has been implicated by the isolation of *c*- or *f*- and *b*-type cytochromes from chloroplasts and from the light-induced absorbancy changes in vivo at wavelengths where cytochromes are known to absorb strongly. In the blue-green alga, *Anacystis nidulans,* light-induced absorbancy changes at 425, about 550, and 556 mμ have been ascribed to oxidation and reduction of *c*- and *f*-type cytochromes (*1*). In the blue-green algae isolation of only a *c*-type cytochrome from *Tolypothrix tenuis* has been reported (*2*). We have isolated from *Anacystis* three water-soluble cytochromes whose properties may have important implications for the interpretation of data obtained in vivo.

Water-soluble cytochromes, along with other pigments, are readily extracted from lyophilized algal cells (*3*). Chromatography of such an extract on diethyl aminoethyl (DEAE)-cellulose permits separation of three cytochrome-containing fractions: (i) cytochrome-552 (*4*) which is not adsorbed on the cellulose and is isolated from that portion of the extract passing through the column; (ii) cytochrome-554 which is adsorbed to the cellulose and is eluted in reduced form at concentrations of about 0.03*M* phosphate buffer, *p*H 7.0; and (iii) cytochrome-549, which is eluted in oxidized form with about 0.09*M* buffer. Cytochrome-552 was partially purified by chromatography on Amberlite XE-64. Cytochromes-549 and -554 were freed of contaminating proteins by chromatography on DEAE-cellulose with elution by *p*H 6.0 and 7.0 phosphate buffers and fractionation with ammonium sulfate, and, in the case of cytochrome-554, an electrophoresis on polyacrylamide gel.

Properties of these cytochromes are summarized in Tables 1 and 2. The