Anaerobiosis and a Theory of Growth Line Formation

Micro- and ultrastructural growth patterns within the molluscan shell reflect periodic respiratory changes.

Richard A. Lutz and Donald C. Rhoads

During the past decade, growth patterns, particularly microstructural increments within the molluscan shell, have been the subject of considerable biological, paleontological, and archeological research (*1–4*). As a result of marked periodicity associated with many of these structures, they have proved useful in geophysical studies for defining Phanerozoic changes in the rate of the earth's rotation (*2*), in ecological and paleoecological studies for assessing the effects of various biological and environmental stresses (*2, 3*), and in archeological studies for reconstructing settlement patterns of prehistoric hunter-gatherers (*4*). Despite this wide range of interdisciplinary applications, hypotheses concerning the formation of periodic growth lines and structures within the molluscan shell are speculative. Some of the studies that are most pertinent to an adequate interpretation of growth patterns within the shell have received little or no attention. We present in this article a coherent theory of growth line and structure formation within the shells of molluscs based on significant advances made during the past decade in the fields of molluscan physiology, shell structure, and growth line research. Terms or phrases within existing literature having genetic connotations which are generally inconsistent with the proposed theory are italicized.

Microstructural Growth Increments

It is accepted that many, if not all, microscopic periodicity structures within the molluscan shell are a reflection of variations in the relative proportions of organic material (conchiolin) and calcium carbonate (aragonite or calcite) (*2, 5*). Alternation of calcium carbonate-rich layers and organic-rich regions or lines has been well documented for numerous Recent and fossil species through optical and electron microscopy studies of thin sections, acetate peels, and polished and etched surfaces of the shell. Such lineations were originally interpreted as reflections of solar time (*2, 5*). Current studies, however, have revealed a complex relationship between *incremental growth* and the lunar and solar cycles. Although a one-to-one correspondence has not been established, the *deposition of increments* in bivalves correlates well with shell valve movements (*2, 6*). Since the valves of many species are usually closed during low tide and open during high tide, a high positive correlation also exists between the number of increments and the number of tides to which an organism has been subjected. While valve-movement rhythmicity is usually most pronounced in intertidal individuals, subtidal specimens of at least one species (*Mercenaria mercenaria*) exhibit biological rhythms in relative harmony with the tidal cycle (*6*). There is general agreement among growth-line workers that when the valves are open and the organism is actively pumping, a layer is deposited that is rich in calcium carbonate relative to the adjacent shell material. Hypotheses attempting to explain the origin of alternating layers or lines relatively rich in organic content fall into three categories. During periods of valve closure the organisms (i) deposit an organic-rich layer or line at the growth surface (that is, calcium carbonate and organic material are deposited at variable rates within tidal or daily cycles) (*2, 5*); (ii) concentrate conchiolin at the growing edge as a result of the juxtaposition of the inner side of the accreting periostracum, where conchiolin has not yet polymerized; or (iii) undergo a period of shell erosion (*3, 7*) or dissolution resulting in a concentration of organic material at the growth surface (*6*).

All the hypotheses above are based largely, if not entirely, on the morphology of the growth structures and the correlation of their *deposition* with various environmental or biological rhythms (or both). Interpretations based on recent studies of molluscan anaerobiosis and mechanisms of shell formation have not been attempted. In the next few paragraphs, we consider the implications of these studies in detail.

Molluscan Anaerobiosis

The ability of molluscs to respire anaerobically has been well documented (*8*). The biochemical pathways operating during anaerobiosis, at least in bivalves, appear different from those described in vertebrates. In contrast to classical vertebrate pathways, in which quantities of lactic acid are produced during anaerobic metabolism, the major end products of molluscan anaerobic respiration are succinic acid and alanine (*9*). Early studies (*10*) suggested that calcium carbonate from the shell served to buffer the acidic products of anaerobic metabolism, and visible erosion of the growth surfaces of *Mercenaria mercenaria* was observed after prolonged periods of shell closure (*11*). The hypothesis has been confirmed through studies of chemical changes in the composition of the extrapallial fluid and measurement of Ca^{45} deposition and solution (*12*); the succinic acid produced during anaerobic metabolism is neutralized by dissolution of $CaCO_3$ from the shell. Furthermore, measurements of the oxygen tension in the extrapallial fluid have demonstrated that *Mercenaria* becomes anaerobic when the valves are closed (*12*). As alluded to by Wilbur (*13*), periodic valve closure should, therefore, result in an alternation of shell deposition and decalcification. Any inclusive theory on growth-line formation within the molluscan shell must account for decalcification at the interface between mantle and shell and its effect on preservation of the *complete record of growth* found within the shells of numerous species (*2, 3*). Ironically, the one species (*M. mercenaria*) in which shell deposition and decalcification cycles have been conclusively demonstrated is a species with a particularly well-preserved record of *growth* within the shell.

The authors are marine ecologists in the Department of Geology and Geophysics, Yale University, New Haven, Connecticut 06520.

Shell Structure Research

The micro- and ultrastructural relations between organic material and inorganic crystals of calcium carbonate within the shells of pelecypods, gastropods, and cephalopods have been well described (*14*). While structures such as interlamellar, intercrystalline, and interprismatic organic matrices are universally recognized, the structural organization of intracrystalline and intraprismatic organic material has been the subject of controversy (*15*). Sometimes this organic material assumes the form of coherent matrices or envelopes within the prisms or aragonitic nacreous tablets, but other studies (*15*) indicate that such organic matter, when present, may be impurities in the form of trapped proteins or polypeptides. While it was originally suggested that such intracrystalline organic matter was water soluble (*16*), subsequent studies have refuted this hypothesis and indicate that the water-soluble fraction appears to be adsorbed on the crystal surface (*14, 17*). The insolubility in dilute acids of much of the organic material relative to calcium carbonate is obvious from an examination of electron micrographs of decalcified shell sections presented by numerous workers (*14, 15*).

Theory of Growth Line Formation

During aerobic metabolism, molluscs deposit calcium carbonate in the form of aragonite or calcite, together with organic material, resulting in shell construction. Such metabolism is usually correlated with periods of active pumping during high tide in well-oxygenated waters. As the concentration of dissolved oxygen falls, such as in the microenvironment created by the organism during periods of shell closure, anaerobic respiratory pathways are employed and the level of succinic acid (or other acidic end products) within the extrapallial fluid rises. The acid produced is gradually neutralized by the dissolution of shell calcium carbonate, leading to increased levels of Ca^{2+} and succinate (or other end products) within the extrapallial and mantle fluids (*12*). As a result of this decalcification, the ratio of relatively acid-insoluble organic material to calcium carbonate increases at the interface between the mantle and the shell. One need not invoke the complication of an increased concentration of organic material in a given volume, although a collapse of unsupported matrix structures or a movement of the mantle as a compensatory response to the increased distance between mantle and shell could result in increased concentrations of freed organic material in specific regions of the extrapallial fluid. With the return of oxygenated conditions and resumption of aerobic metabolism, and assuming that shell deposition during this post-anaerobic period proceeds by a process similar to that occurring immediately prior to anaerobiosis, the deposition of calcium carbonate and organic material within an area already containing organic material should result in an increase in the ratio of organic material to calcium carbonate, within the specific shell region. The end product of this process, from a strictly structural viewpoint, is one growth increment. The presence of intraprismatic and intracrystalline coherent organic matrices would generally be more compatible with this hypothesis than would reincorporation into the shell of organic inclusions freed during decalcification, although the latter interpretation is tenable.

Growth Lines in Prisms

Illustrations of the above process of growth line formation are provided by a reinterpretation of the works of Nakahara and Bevelander (*18, 19*). In their electron microscope study of the formation and growth of the prismatic layer of *Pinctada radiata*, they envisioned fragments of "electron-dense lamellae" at the internal boundary of future prisms as migrating through the extrapallial fluid and ultimately forming envelopes within which crystal nucleation and growth occur. If the micrographs presented are reinterpreted and the described process of prism formation is envisioned as occurring in reverse, we can observe quite vividly the process of gradual shell destruction. As calcium carbonate slowly dissolves, relatively insoluble organic envelopes, matrices, or inclusions remain at the mantle-shell interface, presenting the appearance of an electron-dense lamella. That this organic residue is subsequently incorporated into the shell, as proposed by our hypothesis, is strongly suggested by the presence of "intraprismatic organic strands" approximately paralleling the inner growth surface of the prisms. While details of the treatment of the studied pearl oysters prior to anesthetization were not presented, it is easily envisioned that laboratory conditions or exposure to air (or both) prior to or during the placement of the organisms in magnesium sulfate could have induced valve closure and subsequent anaerobiosis.

Growth Lines in Nacre

Results of an earlier study conducted by Bevelander and Nakahara on the formation and growth of nacre (*18*) have been interpreted as illustrative of shell dissolution (*17, 20*). That their organic "compartments" at the mantle-shell interface may indeed be reflections of shell dissolution is suggested by an examination of the treatment of the studied bivalves prior to fixation. For a period of 1 hour before fixation, the molluscs were placed in a refrigerator at 5°C. As implied earlier (*12*), such treatment is almost certainly sufficient to induce a state of anaerobiosis, and subsequent release of succinic acid to the extrapallial fluid. Neutralization of this acid by calcium carbonate from the shell would be expected to result in selective shell destruction in a manner virtually identical to that seen in their presented micrographs. While microstructural growth increments within nacre have seldom, if ever, been reported (*2, 3*), they do occur. Nonreflected growth lines (*2*) within the middle nacreous layer [inner nacreous layer of previous workers (*21*)] of the Atlantic ribbed mussel *Geukensia (=Modiolus) demissa* (Fig. 1A) show clustering patterns similar to those reported in a number of species (*2, 5, 22*). Mechanisms of growth line formation within nacreous structures may or may not be precisely analogous to the process within the prismatic layer of *Pinctada*. If intracrystalline organic matrices are present, the process may be almost identical to that occurring in *Pinctada* prisms. In light of the current controversy over the presence or absence of such organic structures, we suggest an alternative mechanism of growth line formation in nacre.

It has been proposed that nacreous crystal nucleation occurs on the surface of an organic substratum and that during growth these crystals are enclosed within tight-fitting organic envelopes (*17, 20, 23*). As a result of the lateral fusion of the crystals to form a mature nacreous lamina, adjacent lateral and distal portions of the envelopes merge to become intercrystalline and interlamellar organic matrices, respectively. If crystal nucleation and growth occasionally occur within organic compartments remaining after decalcification, and subsequent growth proceeds as described above, lateral fusion of adjacent crystals should result in a marked increase in the thickness of interlamellar and intercrystalline organic matrices. That this may occur is suggested by the widely variable thicknesses of these structures in micrographs presented by numerous workers (*14, 15*). Such an increased concentration of conchiolin relative to calcium carbonate could explain growth patterns observed on acetate peels of polished and etched sections of *G. demissa* nacre. Evaluation of the above alternation of shell dissolution and deposition in the perspective of the current controversy associated with the *template* and *compartment* hypotheses (*18, 19, 23*) suggests that, while compartments may exist from a strictly structural viewpoint, they are not primary features and should be considered of only incidental importance in the formation of nacre.

Evidence that shell-destructive mechanisms, such as those described above, may be operating in *G. demissa* is provided by analysis of seasonal variation in the shell structure of this species. Mature specimens of *G. demissa* were sampled at monthly and, occasionally, biweekly intervals over a 3-year period, from a natural intertidal population in the Gulf of Maine estuarine waters. Specimens were collected during low tide, the adductor muscles were severed, and soft tissues were removed in the field, taking care not to damage inner shell layer growth surfaces. In the laboratory, the shells were gently rinsed under tap water and allowed to dry in air. Selected regions of the growth surfaces were removed, coated (under vacuum) with gold-palladium or a combination of gold and carbon, and examined under several different scanning electron microscopes (Cambridge S-4, AMR-1000A, ETEC Autoscan).

Earlier investigations have revealed an annual variation in structural patterns within the inner shell layer of *G. demissa*, with nacre deposition restricted to the relatively warm months of the year (*24*). The morphological appearance of the growth surface of the inner layer at various times of the year has not been described. The sequence of events occurring on this growth surface is summarized as follows. During the warm summer months (June through September), very regular hexagonal nacreous tablets are arranged in steplike patterns characteristic of bivalve nacre (Fig. 1B) (*23*). As water temperatures decline, the nacreous tablets become smaller and less regular, showing visible signs of erosion in the form of marked pitting and hollow crystals (*15, 20, 25*), as well as increased proportions of fine-grained structures (Figs. 1C and 2). A similar irregularity of nacreous crystals as a result of dissolution during reduced winter temperatures (as low as 8°C) was found by Wada (*25*) in his examination of growth surfaces of *Pinctada martensii* and *Pinna attenuata*. During the colder months of the year (January to March, with water temperatures below 3°C), shell erosion becomes visible to the naked eye, the entire inner shell surface often pre-

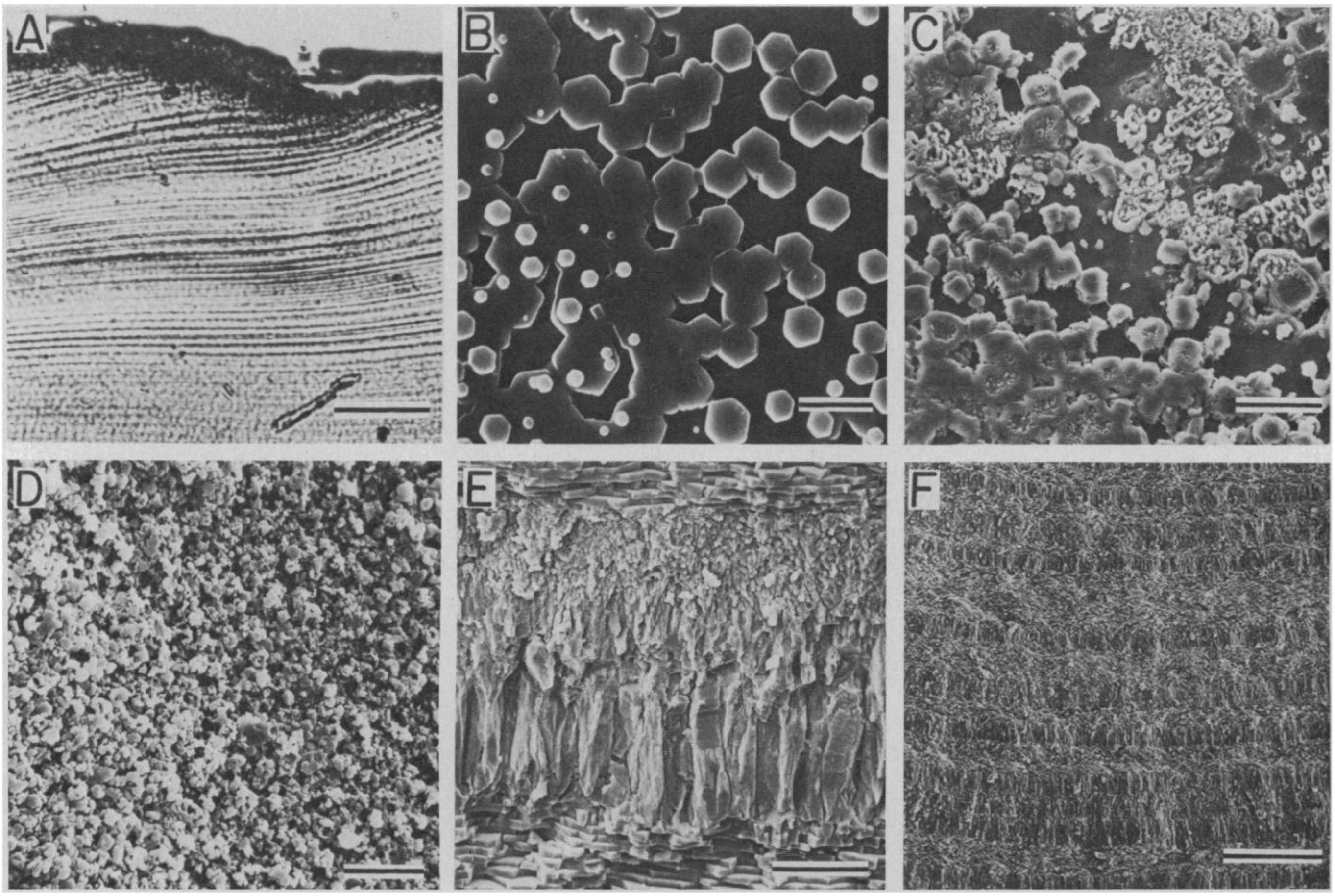

Fig. 1. (A) Optical micrograph of an acetete peel showing nonreflected growth lines within the middle nacreous layer of *Geukensia demissa*. Note the pronounced clustering of increments (scale, 100 μm). (B–F) Scanning electron micrographs. (B–D) Inner shell layer growth surface of *Geukensia demissa* as seen during various months of the year. (B) Regular hexagonal nacreous tablets arranged in steplike patterns characteristic of bivalve nacre (August sample) (scale, 10 μm). (C) Small, irregular nacreous tablets showing visible signs of erosion in the form of marked pitting and increased proportions of fine-grained structures (November sample) (scale, 5 μm). (D) Fine-grained structures reflective of extensive shell dissolution during the colder months of the year (February sample) (scale, 10 μm). (E) Vertical fracture through the inner shell layer of *Geukensia demissa*. Nacreous tablets grade into fine-grained structures at the top of the micrograph and the prisms grade into nacre at the bottom. The most recently deposited crystals are at the bottom of the micrograph (scale, 10 μm). (F) Vertical fracture through the inner shell layer of *Arctica islandica* near the umbo. Bands of simple aragonitic prisms are seen alternating with complex crossed-lamellar structures (scale, 40 μm).

senting a chalky white appearance. Ultrastructurally, this surface appears uniformly fine-grained (Fig. 1D). As we mentioned earlier, similar visible erosion has been reported in *M. mercenaria*, after long periods of valve closure resulting in extended periods of anaerobiosis (*11*). The ability of *G. demissa* to respire anaerobically for extended periods of time has been described (*26*) as has the relative increased efficiency, in this species, of some of the citric acid cycle enzymes in an anaerobic direction (*27*). The observed shell erosion may well be a reflection of the buffering of acidic end products from anaerobic metabolism during the colder months when oxygen transport into the cells should theoretically be reduced relative to that occurring at higher temperatures (*28*). Wilbur (*13*) has suggested that during periods of "adverse environmental conditions," shell decalcification may predominate over growth. The gradation in fractured, as well as polished and etched, vertical shell sections of *G. demissa* nacreous laminae into fine-grained structures (suggestive of massive erosion) instead of regular prisms (Fig. 1E) tends to support this view. As water temperatures rise during the spring, the sequence of events described above is reversed. Examination of growth surfaces during transition periods (spring and fall) between normal nacreous deposition (summer) and drastic erosion (winter), reveal differential dissolution of calcium carbonate and organic material, with eroded aragonitic tablets above and below exposed sheets of interlamellar organic matrices (Fig. 2). Many of the discontinuities (holes) observed within the exposed organic sheets may be artifacts of desiccation. The overall appearance of these structures at the growth surface is similar to that reported by Bevelander and Nakahara (*18*) at the mantle-shell interfaces of the bivalves that they studied. Successive monthly samples often indicate a net gain of shell material during these "transition" periods. We, therefore, suggest that the observed growth surfaces are a reflection of alternating periods (not necessarily rhythmic) of shell deposition and destruction. Here, alternating periods of aerobic and anaerobic metabolism, respectively, which have already been demonstrated to occur in at least one species (*M. mercenaria*) (*6, 12*), could easily provide the driving forces. Similar metabolic changes, perhaps in synchrony with lunar or solar cycles (or both), may be responsible for the formation of microstructural increments within the nacre of this, and perhaps many other, species.

Phylogenetic and Paleoecologic Implications

In previous studies, fundamental differences in the mode of nacre formation between gastropods, cephalopods, and pelecypods have been hypothesized (*20, 29–31*). During "transition" periods in *G. demissa*, the growth surface of the inner shell layer, with its stacked nacreous tablets [*Vertikalschichtung* (*29–31*)], appears similar to that observed in gastropods and cephalopods (Fig. 2) (*20, 29–32*). If the growth surfaces of gastropods and cephalopods, with their irregular and often pitted nacreous crystals, are viewed as reflections of alternating periods of shell destruction and deposition, then complicated fundamental differences in the primary mode of nacre formation between these three classes of molluscs need no longer be hypothesized. "Organic membranes" (*20, 29–32*), previously interpreted as primary depositional structures, need only be viewed as remnants of organic matrices exposed during periods of shell dissolution. Alternating periods of aerobic

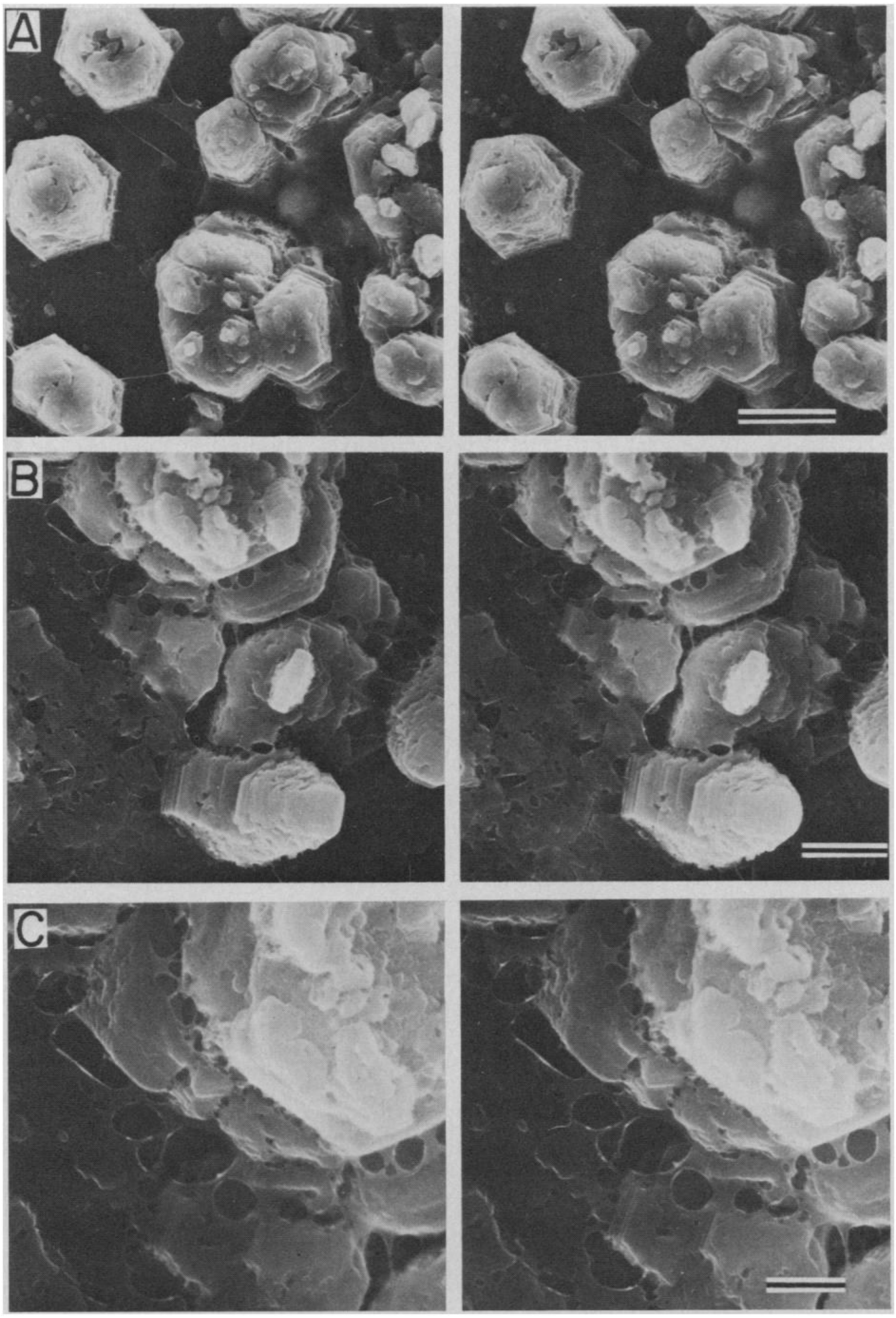

Fig. 2. Scanning electron micrographs of the inner shell layer growth surface of *Geukensia demissa* showing natural shell dissolution. Stereo pairs were taken with a 6° angular displacement between exposures. Note the marked pitting and stacked appearance of nacreous tablets. The differential solubility of calcium carbonate and organic matrices is apparent. Scale bars are as follows: (A) 5 μm; (B) 2 μm; and (C) 1 μm.

and anaerobic respiration, with shell deposition during aerobic metabolism predominating over anaerobic dissolution, could easily account for net shell growth.

It has been proposed that the stacked *mode of deposition* is a primitive trait among molluscan species which has been lost by degrees in pelecypods during their evolution of a "new (bivalve) shell form" (*29–31*). More recently, Taylor (*33*) has suggested that the "vertical component" of nacre is more closely related to the geometry of the shell rather than the antiquity of the lineage, arguing that bivalves with low expansion rates (high convexity) will have better developed columnar nacre than forms with higher rates of expansion (*34*). One may alternatively view the various configurations of nacre within the molluscan shell as reflections of metabolic pathways employed. Assuming gradual increases in atmospheric oxygen levels since the late Precambrian (*35*), we suggest that increased aerobic respiration has resulted in an increased shell deposition to shell dissolution ratio and, hence, increased proportions of sheet nacre (*14*). Moreover, in *G. demissa*, during "transition" periods described above, increased stacking of crystals actually results in structural changes, during which nacre subtly grades into simple aragonitic prisms or the reverse (Fig. 1E) (*36*). Physiological or other mechanisms (or both) responsible for the increased stacking of nacreous tablets and for this gradation of nacre into simple prisms warrants additional research. Similar bands of simple aragonite prisms are found alternating with complex crossed-lamellar and homogeneous structures within the inner shell layer of *Arctica islandica* (Fig. 1F), an organism known to respire anaerobically aperiodically, during extended burrowing activities (*37*). This suggests that such aragonite prisms may represent a shell microstructure that reflects low oxygen environments. Such a view is compatible with the phylogenetic tree presented by Taylor *et al.* (*38*) in which the relationships between bivalve superfamilies, their possible history, and their shell structures have been summarized. Here, the earliest ancestral forms in which shell structures can be recognized are composed of nacre and simple prisms of aragonite (*39*). Similarly, Carter and Aller (*40*) hypothesized a gradual evolution of molluscan shell structures from a primordial shell plate with simple prismatic structure. We suggest that the *evolutionary scheme* proposed by Wise (*29, 30*) be extended backward one step. Using the terminology of Schmidt (*31*), we propose a gradual change (not necessarily evolutionary in a genetic sense) within certain ancestral molluscan lines from simple aragonitic prisms to *Vertikalschichtung* (stacked or lenticular), then *Treppen* (stepped), and, finally, *Backsteinbau* (sheet or brick-wall) nacre (*41*). Inasmuch as such structural changes may be correlated with oxygen tension, a detailed analysis of relationships between ambient oxygen concentrations and various shell *structural types* within Recent and fossil molluscan superfamilies may eventually prove useful, in paleoecological studies, for the determination of dissolved oxygen gradients in ancient marine environments.

Summary

Microstructural growth increments within the shells of numerous Recent and fossil molluscs are interpreted as reflections of alternating periods of shell deposition and dissolution, occurring during aerobic and anaerobic respiration, respectively. The acidic end products of anaerobic metabolism are neutralized by calcium carbonate from the shell, leaving a relatively insoluble organic residue at the mantle-shell interface. With the return of oxygenated conditions and resumption of aerobic respiration, this organic material is reincorporated within the shell. Inasmuch as metabolic changes are often synchronized with lunar or solar cycles (or both), we are led to the nearly paradoxical conclusion that, as a result of shell destructive processes, a relatively complete and detailed record of both short- and long-term growth is often preserved within the molluscan exoskeleton. Analyses of relationships between ambient oxygen concentrations and shell *structural types* may eventually prove useful, in paleoecological studies, for determination of dissolved oxygen gradients in Phanerozoic marine environments.

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42. We thank J. D. Taylor, J. D. Hudson, C. MacClintock, M. A. Crenshaw, K. K. Turekian, K. M. Waage, R. A. Berner, J. G. Carter, M. R. Carriker, D. Jablonski, and R. E. Dodge for discussions and critical reviews of the manuscript. We thank A. S. Pooley, E. Tveter Gallagher, and A. Krishnagopalan for technical assistance with the scanning electron microscopy, W. C. Phelps for preparation of specimens, and W. K. Sacco for his assistance in photograph reproductions. Supported in part by NOAA grants 04-6-158-44056, SGI-77-17, and 04-7-158-44034 and EPA grant R804-909-010. Contribution 106 from the Ira C. Darling Center, University of Maine, Walpole 04573.

NEWS AND COMMENT

Creative Penmanship in Animal Testing Prompts FDA Controls

Inaccurate science, sloppy science, fraudulent science—these are the greatest threats to the health and safety of the American people. Whether the science is wrong because of clerical error, or because of poor technique, or because of incompetence, or because of negligence, is less important than the fact that it is wrong. For if it is wrong, and if the FDA did not know it was wrong, then the protective regulatory barrier between a potentially dangerous drug and the patient is removed.—SENATOR EDWARD KENNEDY (D-Mass.), in congressional hearings on preclinical testing.

In the wake of recent evidence of massive deficiencies in scientific data that were crucial for the approval of hundreds of chemicals and drugs now used in the United States, the federal government is about to impose sweeping new rules for the conduct of laboratory testing on the safety of such products.

The new rules, which are known as the Good Laboratory Practice (GLP) regulations, to be imposed in January by the Food and Drug Administration (FDA), will cover nearly every facet of the operation of nonclinical, or animal, laboratories, from the care and feeding of test animals to the storage and retrieval of raw scientific data. All types of testing for toxicity with animals, whether to determine the potential of a substance to cause birth defects, cancer, mutations, or degenerative disorders, are likely to be covered by the rules.

Nearly 400 corporations, contract laboratories, and universities—each of which provides information to the FDA in support of the safety of a new food or drug—will be affected. Several parts of the new requirements are regarded as so strict that universities either will not want to comply with them or will not be able to afford compliance, and thus will be excluded from the lucrative market of testing for toxic effects of regulated products. A study that found university labs to be the worst performers of the tests has killed chances that their labs would be excused from compliance.

Industry officials have forecast that the regulations will increase the cost of such testing by at least 20 percent, and even the FDA places the overall cost for their implementation at each testing facility at an average of $150,000. It is no surprise, then, that the proposed GLP's have not been kindly received. Nearly 200 comments, almost all of them negative, were sent to the FDA by laboratory researchers and industry officials from every corner of the United States, as well as from France, England, Germany, and Belgium.

The volume and vehemence of this opposition are tangible indications that the GLP's constitute a major new initiative for the FDA. Until a short time ago, the agency's efforts to ensure the authenticity of test data it received were restricted to audits and lab inspections initiated only after an employee in one of the agency's divisions spotted something unusual in the report provided to the FDA by the corporate sponsor of a new product. According to Ernest Brisson, the associate director for compliance in charge of the FDA's new Bio-research Monitoring Program, the limited scope of these audits and inspections reflected an "assumption that the conduct and findings of studies submitted to the Agency represented scientific research of the highest quality. Reports were assumed to be accurate accounts of well-controlled scientific studies, and we, therefore, made our regulatory decisions accordingly."

Then, after an improbable series of events (see box), FDA investigators discovered, in Brisson's tactful words, "that the scientific integrity of some individuals and establishments engaged in research is open to question." Specifically, in three notorious cases presented in 1976 to Senator Edward Kennedy's Subcommittee on Health, massive deficiencies were found in scientific data submitted to FDA and the Environmental Protection Agency (EPA) by G. D. Searle & Co., of Skokie, Ill., Biometric Testing, Inc., of Englewood Cliffs, N.J., and Industrial Bio-Test Laboratories, Inc., of Northbrook, Ill. According to Brisson, FDA inspectors found that in several instances, gross lesions in test animals were not properly examined or reported to the FDA, and experiments were designed in such a way as to obscure whatever toxic effects the products may have had. "We also encountered creative penmanship which causes test animals to appear and disappear throughout the course of a study," he said, "[circumtances that] make us wonder who is running the show, a toxicologist or a magician."

Such findings are particularly disturbing in light of the fact that in the Industrial Bio-Test (IBT) case alone, tests were submitted that led to the approval of nearly a hundred products by the FDA and 123 pesticide ingredients by the EPA. IBT data also were relied upon by the EPA and ultimately the corresponding agencies of several foreign countries, in setting the accepted levels of tolerance in foodstuffs for 160 pesticide products. So pervasive are the deficiencies, according to FDA and EPA officials, that every one of the thousands of tests re-