lower mandibular boundaries described by homologous parts of respective symmetric axes are nearly parallel in this region. Gross anatomic variations in the dysostotic mandibles preclude a simple analysis of this type for these data.

Considered in toto, these data confirm that symmetric-axis analysis can be used (i) to quantify shape properties of the human mandible, (ii) to make longitudinal comparisons of shape of individuals having specified developmental anomalies, and (iii) to determine angular invariants in the lateral shape of the human mandible, some of which appear to be relatively independent of age both within and between individuals.

The mechanism responsible for these findings is unknown. Some investigators hold that skeletal development is regulated largely by genetic determinants, while others believe that the play of mechanical stresses is the dominant influence (*7*). More likely both mechanisms interplay in a complex fashion. The remarkable stability of these angles with age suggests that they may correspond to genetically influenced discontinuities analogous to branches of a tree near their origin. Bifurcation angles are relatively independent of age, even though the direction a branch may take shortly after division is heavily influenced by a host of environmental factors. If this analogy holds, we would expect analysis of branch points and symmetric-axis angles in other skeletal systems amenable to accurate registration in two dimensions to yield comparable findings.

RICHARD L. WEBBER
National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20014

HARRY BLUM
Division of Computer Research and Technology, National Institutes of Health

References and Notes

1. H. Blum, *Ann. N.Y. Acad. Sci.* **231**, 19 (1974); *J. Theor. Biol.* **38**, 205 (1973).
2. _____ and R. Nagel, *Pattern Recognition* **10**, 167 (1978).
3. B. H. Broadbent, Sr., B. H. Broadbent, Jr., W. H. Golden, *Bolton Standards of Dentofacial Developmental Growth* (Mosby, St. Louis, 1975).
4. F. G. Roberts, S. Pruzansky, H. Aduss, *Arch. Oral Biol.* **20**, 265 (1975); S. Pruzansky, *Birth Defects Orig. Artic. Ser.* **5**, 120 (1969).
5. P. V. de Souza and P. Houghton, *Comp. Biomed. Res.* **10**, 333 (1977).
6. R. L. Webber, H. Blum, R. N. Nagel, *Int. Assoc. Dent. Res. Abstr.* **57**, 112 (1978).
7. D. H. Enlow, *Handbook of Facial Growth* (Saunders, Philadelphia, 1975).
8. We thank R. N. Nagel, M. O'Conner, and M. Nashman for the computer implementation of this project; R. Christiansen, S. Pruzanski, and H. Aduss for the cephalometric data evaluated in this study; and J. Mosimann for his guidance and helpful editorial suggestions.

10 May 1979; revised 11 July 1979

A New Mineralized Layer in the Hinge of the Oyster

Abstract. *A discrete, calcareous layer that binds the foliated calcite of the oyster's shell to the organic ligament in the hinge is reported, apparently for the first time. This layer is ultrastructurally, and generally mineralogically, different from the underlying foliated calcite, and is named the ligostracum.*

In molluscan bivalves, a thin, discrete, mineralized veneer that is continuous over the outer surface of the valves binds the organic periostracum to the outermost mineralized shell region. This layer, the mosaicostracum, has been described ultrastructurally in tellinid bivalves (*1*) and in the blue mussel *Mytilus edulis* (*2*), and is homologous with a similar layer on the surface of the valves of gastropods (*3*).

The juncture between the mineralized shell and the ligament in bivalves has apparently not been investigated. We report the presence of a thin, distinct, calcareous layer in the hinge of oysters that binds the foliated calcite of the shell in the umbonal region to the organic ligament. We name this layer the ligostracum (L., *ligare*, to bind; Gk., *ostrakon*, shell). It is ultrastructurally, and generally mineralogically, different from the underlying foliated calcite of the shell.

We examined 12 *Crassostrea virginica* oysters. Some came from an estuary in Delaware, some from local controlled maricultural systems (*4*), and others from Florida. They ranged in height from 1.7 to 6.0 cm. We also studied adult specimens of *C. angulata*, *C. gigas*, *Ostrea edulis*, *O. lurida*, and *O. equestris* (Table 1). The umbonal region of each oyster shell was removed with a diamond saw and the ligament was dissolved in sodium hypochlorite (0.5 to 1.8 percent) for 15 to 48 hours at room temperature. Time of immersion and concentration of sodium hypochlorite were determined by shell size. Chondrophoral and nymphal surfaces (Fig. 1, A and B) (*5*) of the hinge on right and left valves were brushed clean with a soft brush, rinsed, and dried. Differentiation of calcite and aragonite was accomplished with Feigl's stain (10 to 30 minutes) rather than with x-ray diffraction because of the thinness of the ligostracum (*6*). Umbonal shell pieces were mounted with silver paint onto aluminum stubs, freeze-dried for 4 days, coated in vacuum with carbon and gold, and then studied with a scanning electron microscope (Philips 501).

Chondrophoral and nymphal surfaces of the hinge of the right valve (Fig. 1B) in all of the oysters we examined were distinctly aragonitic, staining intensely black with Feigl's fluid. However, on the left (attached) valve (Fig. 1A), chondrophoral and nymphal areas were aragonitic only in *O. lurida* and *O. equestris* (Table 1). In the other species the two minerals were heterogeneously distributed on these surfaces. The presence of two $CaCO_3$ polymorphs in a single shell layer is rare in the Bivalvia (*7*). Mixed polymorphs of $CaCO_3$ were also found in other areas of the valves. For example, sawed cross sections of the umbonal region of the valves disclosed prominent strata of aragonite alternating with strata of calcite in *C. virginica* and *C. gigas* and traces of aragonitic strata in the other four species—an observation which may not have been made before for ostreids (*8*). Although artifacts with Feigl's stain are possible (*9*), the intensity and rapidity of the staining tend to confirm the aragonitic nature of these strata. This suggests that the oysters, in response to environmental or physiological changes,

Table 1. Mineralogy of the ligostracum of the left valve in various oysters. The chondrophores and nymphae of the right valve contained aragonite in each case.

Species	Source	Chondrophores	Nymphae
Crassostrea virginica (Gmelin)	Delaware Florida	Calcite with traces of aragonite	Calcite with traces of aragonite
Crassostrea angulata (Lamarck)	England	Aragonite with traces of calcite	Aragonite
Crassostrea gigas (Thunberg)	Washington	Aragonite and calcite mixed	Aragonite with traces of calcite
Ostrea edulis (Linné)	Maine	Aragonite and calcite mixed	Aragonite
Ostrea lurida (Carpenter)	Washington	Aragonite	Aragonite
Ostrea equestris (Say)	North Carolina	Aragonite	Aragonite

0036-8075/79/1109-0691$00.50/0

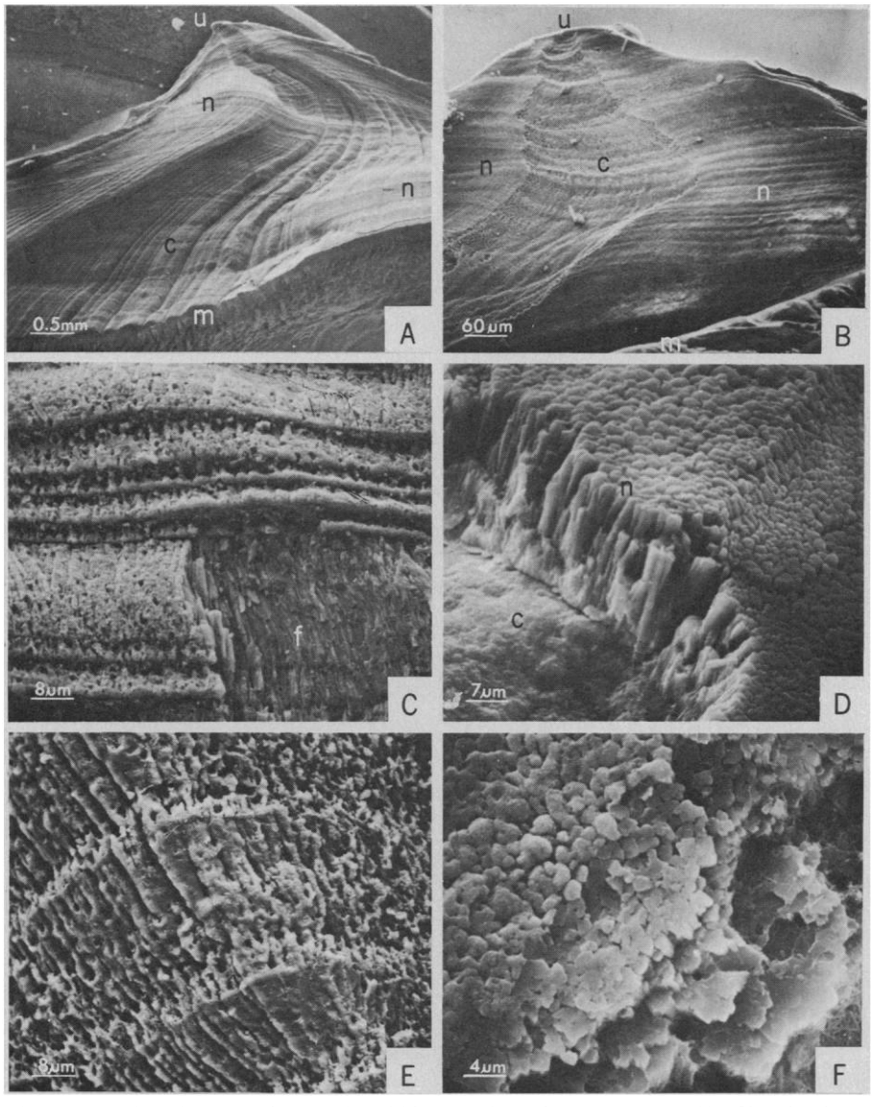

Fig. 1. Scanning electron micrographs of hinge surfaces in *C. virginica*. (A) Chondrophore (*c*), nymphae (*n*), umbo (*u*), and mantle isthmus (*m*) of left valve in an oyster 47 mm high. (B) Chondrophore, nymphae, umbo, and mantle isthmus of right valve in an oyster 19 mm high. (C) Nymphal ligostracum of left valve, showing growth bands and fracture (*f*) of left valve in an oyster 30 mm high. (D) Nymphal ligostracum (*n*) overlying foliated calcite (*c*) of right valve in an oyster 47 mm high. (E) Chondrophoral ligostracum of left valve, showing growth interruption lines in an oyster 52 mm high. (F) Fracture of nymphal ligostracum of right valve, showing successive merging of prisms with depth in an oyster 47 mm high.

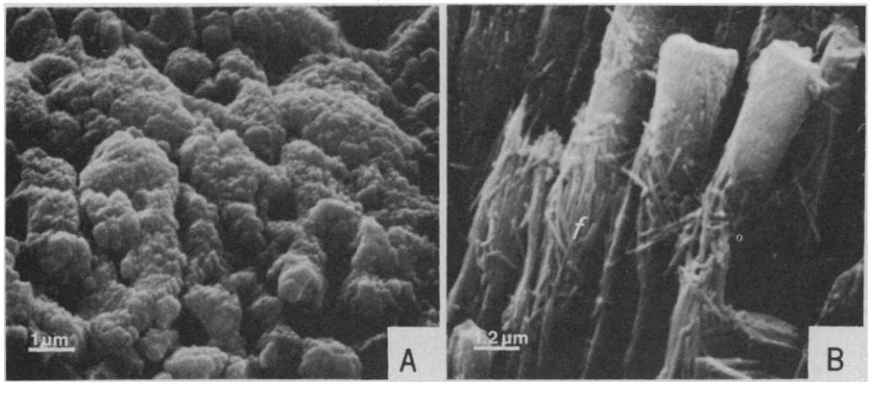

Fig. 2. Scanning electron micrographs of ligostracum in *C. virginica*. (A) Distal ends of nymphal prisms in left valve (against ligament) of an oyster 47 mm high, illustrating finely granular surface and pits among prisms. (B) Fracture of ligostracum parallel to major axis of prisms, showing aragonitic fibers (*f*) clinging to sides of prisms in right valve of an oyster 47 mm high.

periodically deposited patches of aragonite in primarily calcitic shell. Species that showed patterns of mixed aragonite and calcite in the chondrophore of the left valve had the thickest strata of aragonite in cross sections of the shell. Other regions of oyster shell that are known to be aragonitic, such as the myostracum of muscle attachment sites and the larval valves (*10*), also stained black. In older portions of the hinge, where the ligament had worn away through natural abrasion, the aragonitic ligostracum had also been removed, revealing the underlying foliated calcite.

The chondrophore on each valve first forms in the late pediveliger stage (*11*). From a width of about 20 μm, the chondrophore increases in width as the valves grow, forming an annulated, serpentine, spreading gully (*4*) leading from the umbo to the mantle isthmus (Fig. 1, A and B). Soon after settlement of the larva, nymphal surfaces that are elevated above the chondrophore develop to either side of it and likewise widen as the valves grow. The chondrophore and nymphae on the left valve have a rolling topography (Fig. 1A), whereas on the right valve they are smoother and more even (Fig. 1B).

The ligostracum completely covers the chondrophoral and nymphal surfaces of the hinge on both valves of *C. virginica*. It is a thin, prismatic veneer that cements the foliated calcite of the valves (*11*) to both the central inner parts (chondrophores) and the anterior and posterior outer parts (nymphae) of the ligament (*12*), and it differs in ultrastructure on the left and right valves (Fig. 1, C and D, respectively).

Ligostracal prisms of the chondrophore of the left valve tend to lie obliquely to the surface of the layer, range in diameter from a fraction of a micrometer to about 6 μm, and are interrupted periodically by growth lines (Fig. 1, C and E) (*4*). Thickness of this layer averages 8 to 16 μm. Prisms are spaced apart at the prism-ligament juncture and are deeply pitted (Fig. 1E). Ligostracal prisms of the chondrophore of the right valve and of the nymphae of the left and right valves are generally oriented at right angles to the underlying foliated calcite and form a distinct layer that can be removed by fracturing. At the surface of the layer, prisms are spaced slightly apart and tend to merge toward the base of the layer (Fig. 1F). Diameter of the distal ends ranges from a fraction of a micrometer to about 4 μm. In fracture sections the nymphal layer averages 15 μm in thickness and may taper to half this thickness at the edges and increase to 30 μm in the

central part of the chondrophore. The surface of nymphal and right chondrophoral prisms in contact with the ligament is finely granular in appearance (Fig. 2A). Fractures of these ligostracal layers expose bundles of calcified fibers clinging to the sides of the prisms (Fig. 2B).

The finely tuberculous, pitted, spaced arrangement of the distal ends of prisms in the oyster ligostracum strongly suggest that the principal function of this layer is to bind the ligament to umbonal shell. The presence of aragonitic fibers [characteristic of the resilial ligament (*13*)] deep among the prisms (Fig. 2B) supports this conclusion. The ligostracum of the hinge is analogous to the mosaicostracum, which binds the periostracum to the valves (*1*, *2*). We suggest that the functional term ligostracum replace the term mosaicostracum and be used to describe all thin mineralized layers that bind external organic layers, such as the ligament and periostracum, to the underlying mineral shell.

Muscle attachment sites (myostracal prisms) in mollusks are invariably aragonitic (*8*). With the exception of the chondrophore in the left valve of oysters, which often contains calcite, all ligostracal layers we examined were aragonitic (Table 1). It would thus appear that aragonitic prisms may serve the function of attachment of muscular and other organic layers better than calcitic shell units.

MELBOURNE R. CARRIKER
ROBERT E. PALMER
College of Marine Studies, University of Delaware, Lewes 19958

References and Notes

1. G. H. Hamilton, *Veliger* **11**, 185 (1969).
2. M. R. Carriker, *ibid.* **21**, 411 (1979).
3. A. D'Attilio and G. E. Radwin, *ibid.* **13**, 344 (1971).
4. R. E. Palmer and M. R. Carriker, *Proc. Natl. Shellfish. Assoc.* **69**, 58 (1979).
5. P. S. Galtsoff, *U.S. Fish Wildl. Serv. Fish. Bull. 64* (1964).
6. J. D. Milliman, *Recent Sedimentary Carbonates, Marine Carbonates* (Springer-Verlag, Berlin, 1974).
7. J. G. Carter and N. L. Eichenberger, paper presented at the annual meeting of the Geological Society of America, Seattle, 1977.
8. J. D. Taylor, W. J. Kennedy, A. Hall, *Bull. Br. Mus. (Nat. Hist.) Zool.* **3** (Suppl.), 1 (1969).
9. J. G. Carter, in *Skeletal Growth of Aquatic Organisms: Biological Records of Environmental Change*, D. C. Rhoads and R. A. Lutz, Eds. (Plenum, New York, in press).
10. H. B. Stenzel, *Science* **142**, 232 (19 → *ibid.* **145**, 155 (1964).
11. M. R. Carriker and R. E. Palmer, *Proc. Natl. Shellfish. Assoc.* **69**, 103 (1979).
12. E. R. Trueman, *J. Microsc. Sci.* **92**, 129 (1951); G. Owen, E. R. Trueman, C. M. Yonge, *Nature (London)* **171**, 73 (1953); C. M. Yonge, *Pubbl. Stn. Zool. Napoli* **29**, 151 (1957).
13. H. B. Stenzel, *Science* **136**, 1121 (1962).
14. We thank K. Chew and G. Walsh for shipments of oysters and W. Kay for preparing the prints of the figures. Supported in part by a grant from the NOAA Office of Sea Grant (project 04-6-158-44025).

18 April 1979; revised 11 July 1979

Xenopus Liver: Ontogeny of Estrogen Responsiveness

Abstract. *Estradiol-17β stimulates the synthesis of numerous proteins exported into the culture medium by* Xenopus *tadpole liver tissue obtained after stage 50 and throughout metamorphosis to stage 66. Although estrogen-induced vitellogenin can be detected as early as stage 54, it is a minor percentage of the exported proteins until after the completion of metamorphosis. In hepatic tissue obtained after metamorphosis, the hormone evokes the synthesis of vitellogenin specifically without affecting the labeling of other secreted proteins.*

In parenchymal cells of the liver of the African clawed frog *Xenopus laevis*, estradiol-17β (1,3,5-estratriene-3,17β-diol) evokes a well-defined end-point response, the synthesis de novo of vitellogenin, which is the precursor of the major egg-yolk proteins (*1*). Although this estrogen-induced response occurs naturally only in sexually mature females, the normally dormant vitellogenin genes of the adult male hepatocytes can be activated by exogenous estrogen (*2*). Hence, the hepatic vitellogenin genes of male *X. laevis* frogs are not permanently inactivated as a result of differentiation, and the cellular components required for the actions of this female sex hormone are present in their livers. But, when do hepatic cells acquire the ability to respond to estrogen during the course of their development? Identification of responsive and nonresponsive hepatocytes

Table 1. Effect of estradiol-17β on incorporation of ^{35}S-labeled methionine into exported proteins in culture media by liver explants of *X. laevis*. Tadpoles (stages 48 to 65) and juvenile frogs (stage 66 and older) were obtained by gonadotrophin-induced matings of adult frogs. The stage of development of each tadpole was determined by using the method of Niewkoop and Faber (*16*). For each experiment, the livers from 30 to 80 tadpoles at the same stage of development were excised under sterile conditions, pooled in sterile culture medium, and divided into two groups. One group was cultured in the continuous presence of estradiol-17β (10^{-8}*M*). To the control group, an equal volume (10 μl) of the hormone solvent propylene glycol was added. [The presence or absence of estradiol-17β is indicated by (+) or (−), respectively.] Livers from juvenile and adult frogs were processed in a similar manner, except that they were cut into 1-mm cubes before being cultured. After culture for 7 days with daily changes of the media (2 ml per group), the hepatic explants were placed in fresh medium containing ^{35}S-labeled methionine (Amersham Corp.; initial specific activity, 250 to 935 Ci/mmole) to label the proteins. The isotope was added to give a final specific activity of 4 Ci/mmole. The culture medium and the conditions for the culture and for the labeling of proteins were the same as those described by Wangh and Knowland (*5*). After a 7-hour incubation, the media were centrifuged at 1000*g* for 15 minutes to remove free cells. Triplicate portions (100 μl) of each medium (*5*) were taken from each culture to determine acid-insoluble incorporation into the secreted proteins (*17*).

Source of explant		Treatment [estradiol-17β (10^{-8}*M*)]	[^{35}S]Methionine incorporation (count/min per 100 μl)*	Percent of control value
Tissue	Developmental stage			
Liver	49 to 50 (tadpole)	+	2,507 ± 262	102
		−	2,457 ± 415	
Liver	51 to 52 (tadpole)	+	32,557 ± 4,887	307
		−	10,571 ± 2,183	
Liver	53 (tadpole)	+	49,922 ± 7,259	594
		−	8,404 ± 2,212	
Liver	54 to 55 (tadpole)	+	11,272 ± 742	299
		−	5,649 ± 75	
Liver	58 to 59 (tadpole)	+	35,350 ± 6,399	1,125
		−	3,141 ± 540	
Heart	53 (tadpole)	+	0	
		−	0	
Eye	53 (tadpole)	+	410 ± 132	96
		−	424 ± 116	
Liver	66 + 7 days (juvenile frog)	+	29,457 ± 1,308	164
		−	17,947 ± 978	
Liver	66 + 4 days (juvenile frog)	+	6,102 ± 175	174
		−	3,494 ± 122	
Liver	66 + 4 days (juvenile frog)	+	10,545 ± 620	180
		−	5,830 ± 386	
Liver	Adult	+	27,642 ± 1,863	258
		−	10,710 ± 594	
Liver	Adult	+	5,784 ± 334	311
		−	1,856 ± 176	
Liver	Adult	+	29,780 ± 1,367	183
		−	15,352 ± 457	

*Mean ± standard deviation.

0036-8075/79/1109-0693$00.50/0