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This volume is dedicated to Dr. Rainer Zangerl

Tooth Histology and Ultrastructure of a Paleozoic Shark, *Edestus heinrichii*

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INTRODUCTION

Edestus heinrichii (Newberry and Worthen, 1866), is a Paleozoic shark known from symphyseal tooth isolates and several articulated tooth bars. Specimens attributed to this genus have been described from Russia, Australia, England, and the mid-continental United States. *E. heinrichii* is one of 15 species within genus *Edestus* that have been distinguished by variations in the dentition size and morphology. Teeth remain the only anatomic evidence of the genus thus far described. This paper re-examines the symphyseal dentition based on new material from the Pennsylvanian shales of the Illinois Basin. Aspects of histology, tissue ultrastructure, tooth ankylosis, gross morphology and embryology of the fossil are examined. Evidence is provided for the absence of orthodentine in the symphyseal teeth. This is the first elasmobranch known to have this condition. The teeth are composed of only two types of dentine: enameloid and trabecular. The ultrastructure of the denteon in

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trabecular dentine is shown to share a similar fundamental structure with the osteon of secondary bone. The specimens studied here are Field Museum of Natural History (FMNH) PF 2848 and PF 2849, of *E. heinrichii.* They are from the Pennsylvanian shales of Mecca Quarry in Parke County, Indiana, collected by Dr. Rainer Zangerl. Recent material for comparison of tissue structure is from *Sphryna tudes* and *Isurid* sharks, from the Field Museum's Department of Fishes.

MATERIALS AND METHODS

The Field Museum study collection has at least 27 individual teeth of *E. heinrichii* so far identified by X-ray, including five partial tooth whorls of from two to three teeth and one completely articulated whorl of nine teeth (fig. 1). One of the partial tooth bars of three articulated teeth with complete crowns and almost complete roots was chosen for sectioning, along with a single isolated tooth. The fossils remained completely embedded in shale and were identified by x-ray (pl. 1). In PF 2849, the anterior teeth were cut serially at 2 mm. intervals into 16 sections and light microscope slides were hand ground (fig. 2). Serial sections 6, 7, and 12 did not survive the mounting and grinding process and fragments of them were used for electron field emission scanning. These fragments were put through successive 24-hr. periods in propylene oxide until the embedded epoxide resins were removed, then dehydrated in absolute alcohol. Some of the specimens at this stage were etched with hydrochloric acid, then air dried. Dried material was mounted on aluminium discs and then coated with gold:palladium (40:60) in an Edward vacuum coating machine. The scans were made by the senior author and by Dr. John M. Clark of the University of Chicago Pritzker School of Medicine on the Hitachi HFS II scanning electron microscope, established by a grant from the Sloan Foundation, at the Enrico Fermi Institute. The scans were done under PHS Grant No. 5 TO5 GMO1939 from the National Institute of General Medical Science.

CONDITION OF THE FOSSILS AND THEIR PRESERVATION

The hard tissues were almost perfectly preserved in the fossilization process. Both tooth specimens were laid down parallel to the shale's bedding plane, as is the case with the vast majority of the specimens in the study collection. X-ray photographs of similarly embedded specimens were made at various angles and checked for

angular deformation; none was found. The x-rays (pl. 1) represent fully sagittal views. Zangerl and Richardson (1963, p. 181) report that a large cladodontid tooth from the same quarry was embedded upright and showed no evidence of distortion due to compression. The shape dimensions are in complete agreement with teeth embedded laterally. Plastic deformation is therefore negligible.

Diagenesis has only slightly modified the morphology and histology. The teeth are to some extent decalcified and bituminized. The burial sediments and diagenetic replacement materials have naturally stained histologic areas uniformly and consistently. Microscopic cavities are neatly stained with iron which is brown to red to orange in transmitted light. On PF 2848, calcite has filled the basal canals and made them opaque, and filerite (zinc sulphate) has formed between the denticles along the borders (pl. 1). The presence of filerite from decomposition is common in the Mecca fossils (Zangerl, pers. comm.).

The depositional environment of the black shales was so acid the bacterial degradation was not very destructive. This permitted a slow steady impregnation with hydrocarbons, a condition most favorable to preservation.

Cracking of the enameloid surface is grossly visible when matrix is removed from the crown. The cracks occur at regular intervals remaining fairly equidistant and run from the base of the crown to the tip (fig. 1). In sagittal view cracks in the trabecular dentine lining the crown perforate the enameloid and open onto the crown surface (pl. 4a). The openings are 40-50μ wide and average .4 to .5 mm. in depth. Electron scans of the enameloid surface (pl. 4b, c) demonstrate that micro-cracks occur at intervals corresponding to the channels in the brightfield views. There is no indication from the examination here that these are anatomic structures. They do not appear to be in association with the vascular pattern of the trabecular dentine they penetrate. Zangerl found in gross examination that the system of macro-cracks is arranged stress-coat fashion and probably resulted from pressure of the burial mud when it lost its plasticity (Zangerl and Richardson, 1963, p. 181). The cracks are presumed to be diagenetic rather than anatomic.

GROSS MORPHOLOGY, VASCULARIZATION, AND ANKYLOSIS

The tooth base presumably grows continually in a longitudinal direction from the time the crown comes into place functionally

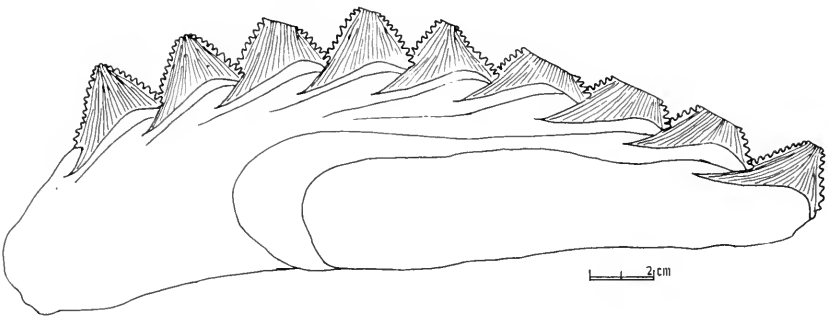


FIG. 1. *Edestus heinrichii*, UF 30 (FMNH), showing a complete symphyseal tooth bar of nine successive teeth. Enameloid flanges can be seen extending posteriorly; the stress-coat-like cracks in the enameloid are approximated.

until the whole tooth including its base is shed from the anterior-most position on the whorl. The crown is full-sized when it comes into place in the posterior-most position. The replacement-shedding process proceeds at a constant rate so that seven to nine teeth are maintained on each bar. The tooth crown is defined by the area covered with enameloid. The cusp is non-equilateral; the anterior edge rises at a sharp angle to the root; the posterior edge slopes at a wider angle. There are up to 11 denticles on the anterior crown border of the adult tooth and 13 along the posterior border. Crenula-

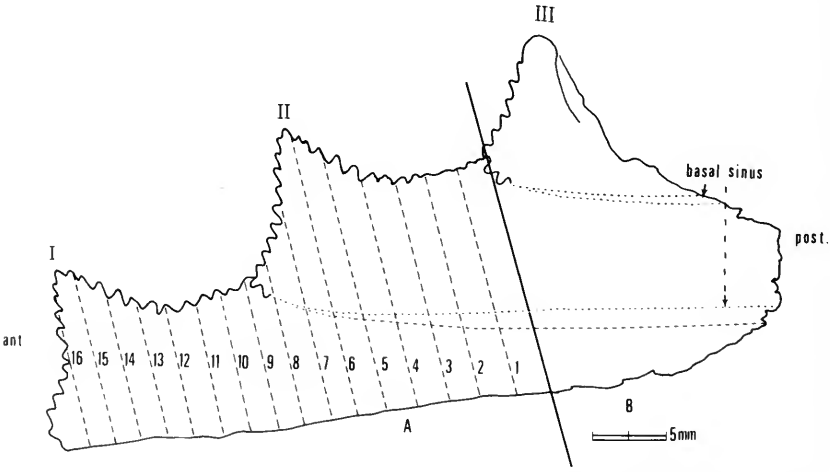


FIG. 2. *Edestus heinrichii*, PF 2849, showing position of coronal sections (A) seen in Plate 2, and the position of the sagittal section (B). The basal sinus seen in the serial sections is approximated by dotted lines.

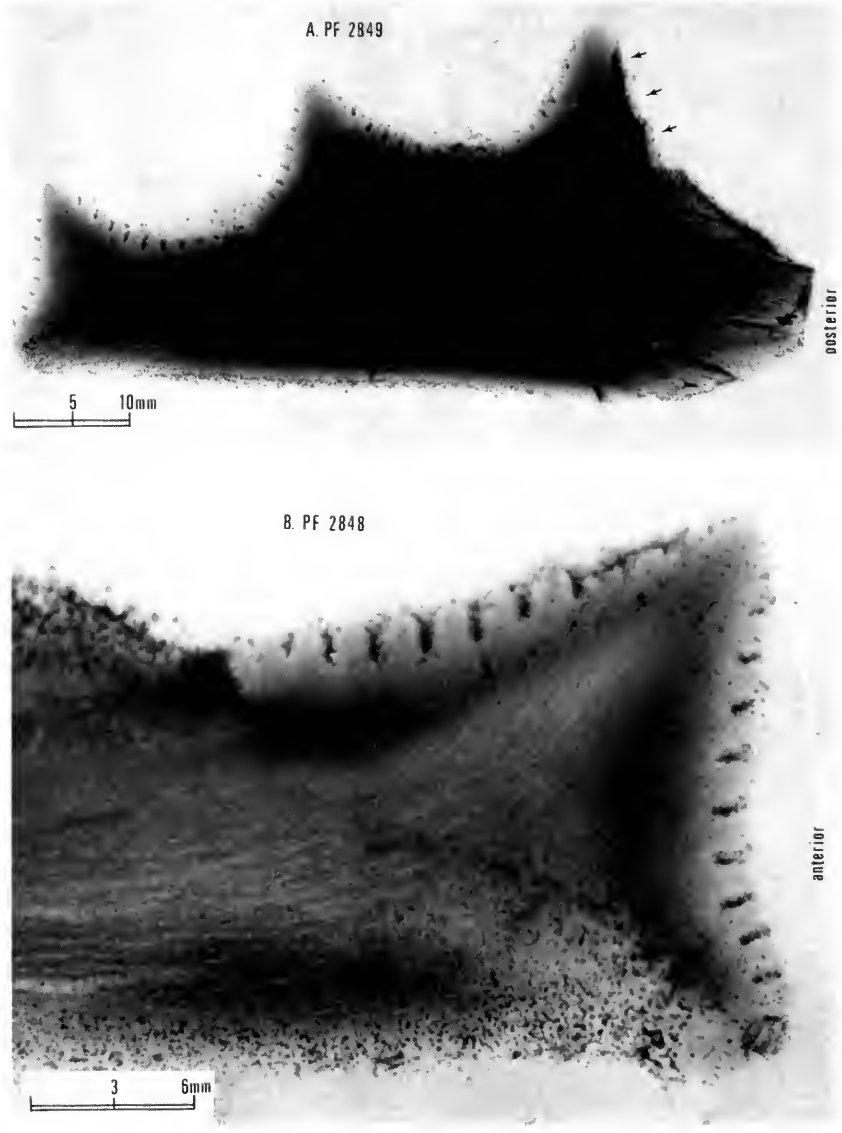


PLATE 1. a, Sagittal X-ray of *Edestus heinrichii*, PF 2849. Three adult symphyseal teeth are seen in anatomic articulation. The arrows along the posterior border of the third tooth indicate a radio-opaque area of pyrite. Filerite, a decomposition phenomenon, has formed between the denticles. **b**, Sagittal x-ray of *E. heinrichii*, PF 2848, is an isolated tooth that was shed anteriorly from tooth bar.

tions on the denticles are not apparent on x-ray but can be seen under magnification on exposed specimens of *E. heinrichii.* The denticles along at least the anterior border are crenulated. The crowns are so closely spaced that the adjacent borders overlap all in the same direction (fig. 1). Flanges of enameloid extend out 1.5 cm. behind the crown on the top of the tooth base troughs (fig. 1) and occur symmetrically on each side of the bar. These flanges also occur in *Edestus minor*, although considerably reduced.

The teeth are well vascularized. The pattern is characterized by major arterial branching and venous anastomosing throughout the tooth base, with substantially smaller arterioles supplying the central crown region and a finer nutrient network going to the apical lining and terminating at the enameloid junction. The vessels run along the longitudinal axis of the root from back to front, diminish in size from frequent branching, and slant upward into the crown. The vessels do not converge toward the crown's apex but remain at right angles to the posterior border as can be seen in a sagittally sectioned tooth (pl. 3b). The largest canals are centrally placed in the root. In the central vascular network there is clearly a single channel that is the major arterial and venous supply for each tooth. Karpinsky (1899, pp. 404-421) described the presence of similar large single channels in *Helicoprion* without discussing their function. The channels' successive branching is clearly demonstrated on the serial enlargements (pl. 2). The central canal slants upward toward the crown and runs in this specimen just to one side of the midline. The canal may conduct arteries, veins, and nerves as is the typical vertebrate circulatory and innervation pattern.

The trabeculation of the tooth bases is more rugged on the exposed outer surface of the tooth bar. This is particularly noticeable on Plate 3a of the serial sections. The external and internal root surfaces facing into the troughs have much smaller trabecles indicating less stress between teeth than between the whorl and the jaws. These internal areas of ankylosis have uniform surfaces and emissary foramina (pl. 3a).

The nature of the ankylosis of the teeth to one another has not been fully detailed before. In his schematic drawing of what he called "*Protopirata heinrichii*" C. R. Eastman (1902) reproduced the presence of a basal sinus which he does not name or discuss. It has otherwise been assumed that the tooth bases were fully in contact with each other (Newberry, 1889; Hay, 1910). This was not found to be the case here. The trough of a tooth base and the base of

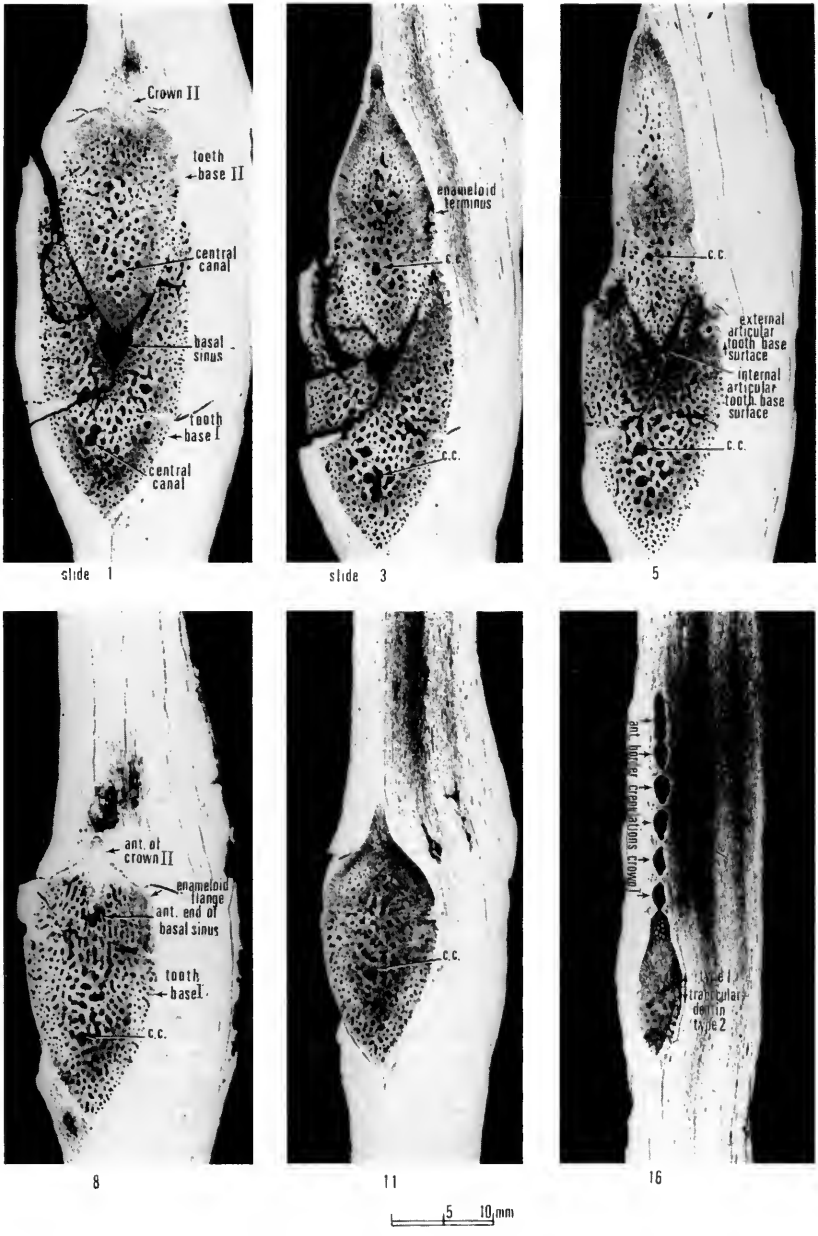


PLATE 2. Coronal serial section of slides 1, 3, 5, 8, 11, 16. Slides 1, 3, 5, and 8 show two articulated teeth. The slides show the course of the central canal vascular supply, demonstrate the basal sinus between articulated teeth, and show the posterior extension of the enameloid flange on the crown.

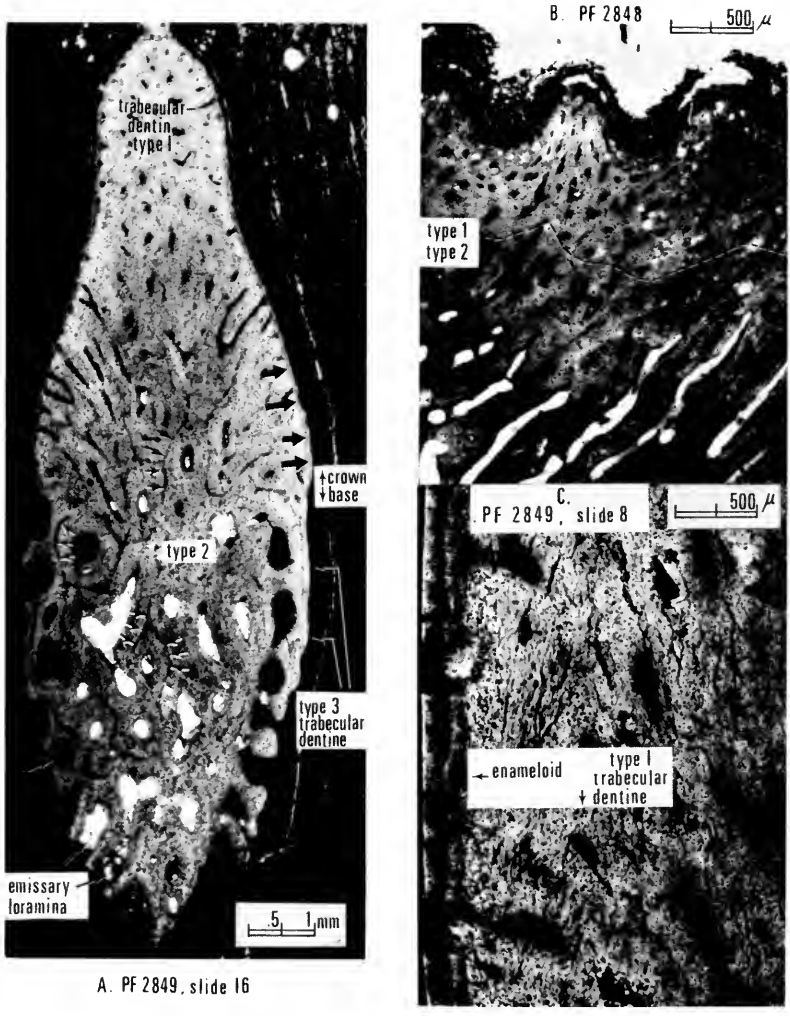
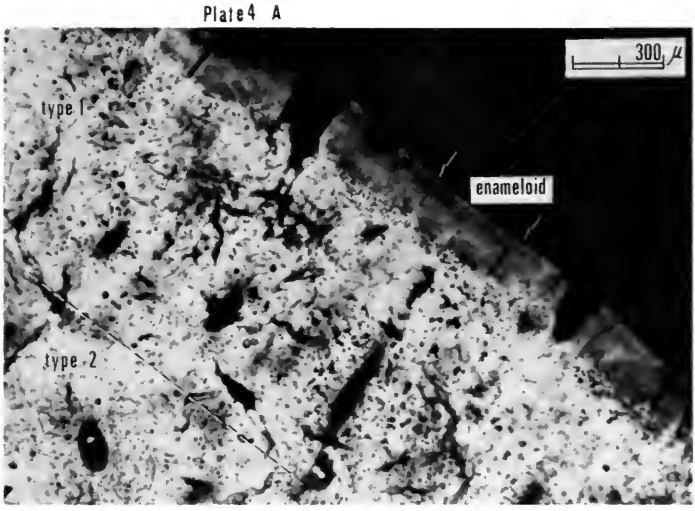
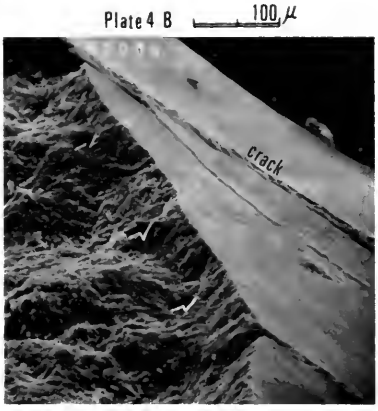


PLATE 3. **a**, PF 2849, coronal section of tooth showing distinction between crown and base. Crown is covered by thin enameloid (see wide arrows), and is composed of Types 1 and 2 trabecular dentine. Interdenteonal hard tissue characterizing Type 2 is shown by thin arrows. Type 3 trabecular dentine is restricted to the outer millimeter of the base and is an open spongiosum lacking denteons. Emissary foramina in Type 3 are associated with rough ligamentous attachment (the trabecles), and with the vascular supply (the foramina). **b**, PF 2848, the vascular pattern in this sagittally cut fossil tooth shows that the vasculature within the denteon lumen run perpendicular to the surface in Type 1 trabecular dentine, and at right angles to the tooth surface in Type 2. **c**, PF 2849, at higher magnification the absence of orthodentine is demonstrated. Type 1 trabecular dentine is subjacent to the enameloid. Here again the regular stress-coat cracking in the enameloid can be seen.





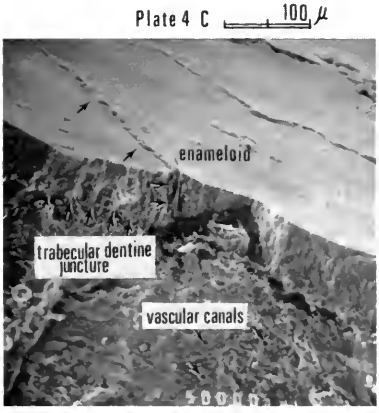


PLATE 4. PF 2849. **a**, Enlargement of the crown tip showing cracks from the enameloid perforating the adjacent trabecular dentine. **b**, **c**, Electron scans of the cracks do not show them to be in association with the vascular pattern. There is a distinct difference in fracture pattern between hypermineralized enameloid and the more fibrous trabecular dentine. The juncture between the tissues shows up clearly.

the successive tooth it holds are not completely ankylosed forming a basal sinus (fig. 2). The sinus is patent only between adjacent tooth bases and is not a continuous channel throughout the intermandibular whorl. The successive basal sinuses are not artifacts of this particular fossil nor a result of the specimens having partially rotted apart. Tracings from blow-ups were cut out and a reassembly attempted that would close off the basal sinuses. Such a realignment was not structurally possible. The basal sinus is a real anatomical feature. There may have been more mobility between teeth than had been supposed with the basal sinus tissues cushioning compressive and shearing stresses, a condition also more conducive for anterior tooth shedding.

HISTOLOGY

Remarkable conservatism in the retention of tooth types is a subclass character of elasmobranchs. This conservatism over a long stratigraphic sequence seems to be the case for the 110-million-year span of *Edestus*, from the Mississippian through the early Triassic. *Edestus* was a successful form. Only two types of dentine — trabecular dentine and enameloid — occurred in its symphyseal teeth. It is the first shark for which the lack of orthodentine has been documented (pl. 4a).

In the early literature terms for different dentine types proliferate that were often defined differently by individual researchers. Ørvig's (1951, 1967a, c) consolidation and reordering of terms for the hard tissues of elasmobranchs is followed here with one exception. Trabecular dentine is used here for what would ordinarily be called osteodentine. We have not been able to identify the interstitial acellular banding between the denteons as bone. Osteoblasts may in certain instances transform into odontoblasts (Pflugfelder, 1930), but invoking such a process without evidence is unwarranted here.

The histology of edestid teeth has been examined previously (Hay, 1910; Nielson, 1932, 1952; Zangerl, 1966). Hay made sagittal and coronal sections of only the tooth base of *E. heinrichii*, therefore not observing the absence of orthodentine in the crown. His specimen came from the same general area, western Indiana, as those examined here. The two correspond exactly in tooth base structure. Hay refers to the trabecular dentine of the base as "vasodentine," a tissue containing capillary canals instead of dentineal tubules; and from gross rather than histologic examination reports that the tooth crown covering "is probably true enamel" (Hay, 1912, p. 50).

Zangerl (1966) described the histology of the closely related edestid *Ornithoprion hertwigi.* The outermost layer, "which probably constituted the orthodentine with its vitrodentine surface" (Zangerl, 1966, p. 31), was missing. In light of its absence in *E. heinrichii* it was probably originally absent in *O. hertwigi* also (Zangerl, pers. comm.). A section through the trabecular dentine of a large *O. hertwigi* tooth shows trabecular dentine corresponding exactly to the type 1 (see pl. 3a, b; 4a) crown lining found in *E. heinrichii.* The clear interstitial banding of acellular calcified tissue is absent, as it is in *E. heinrichii*, and the dentine tubules do not define the denteon margins.

Dentine is homologous among all vertebrates, the matrix being secreted by mesodermally derived odontoblasts. The odontoblasts retreat along the front of the matrix accumulation, leaving hair-like cell processes, called Tomes' fibers, behind (pl. 5b). Orthodentine is the same histologically in fish, reptiles, and mammals. Its absence in this species and probably the whole family is a feature for which there is no ready explanation. Peyer (1968, p. 65) emphasizes that in all known elasmobranchs, both fossil and extant, the outermost coat of compact dentine is orthodentine. It is undoubtedly lacking in *E. heinrichii.* Some elasmobranch teeth consist almost entirely of orthodentine and there is a transition to teeth of very largely trabecular dentine with orthodentine forming a very thin coating. *E. heinrichii* is interpreted here as an evolutionary form in which the tendency toward reduction of orthodentine has culminated in its complete absence. Holocephalians characteristically lack orthodentine also; this is not to suggest that *Edestus* is more closely related to them than to elasmobranchs, but that this is a feature of convergent evolution.

TRABECULAR DENTINE HISTOLOGY AND ULTRASTRUCTURE

Three morphological types of trabecular dentine were found at the light-microscope level. The tissue is one of the most widely distributed hard tissues in early elasmobranch teeth with the same histologic and ultrastructural characteristics abundantly represented in modern sharks. All three morphological types are seen in a single tooth organ. Type 1 trabecular dentine is a dense packing of denteons enclosing a fine capillary system lining the tooth crown (pl. 3a, 4a). There is diagnostically no interstitial tissue between the denteons in Type 1 and the calcified peritubular lining is much re-

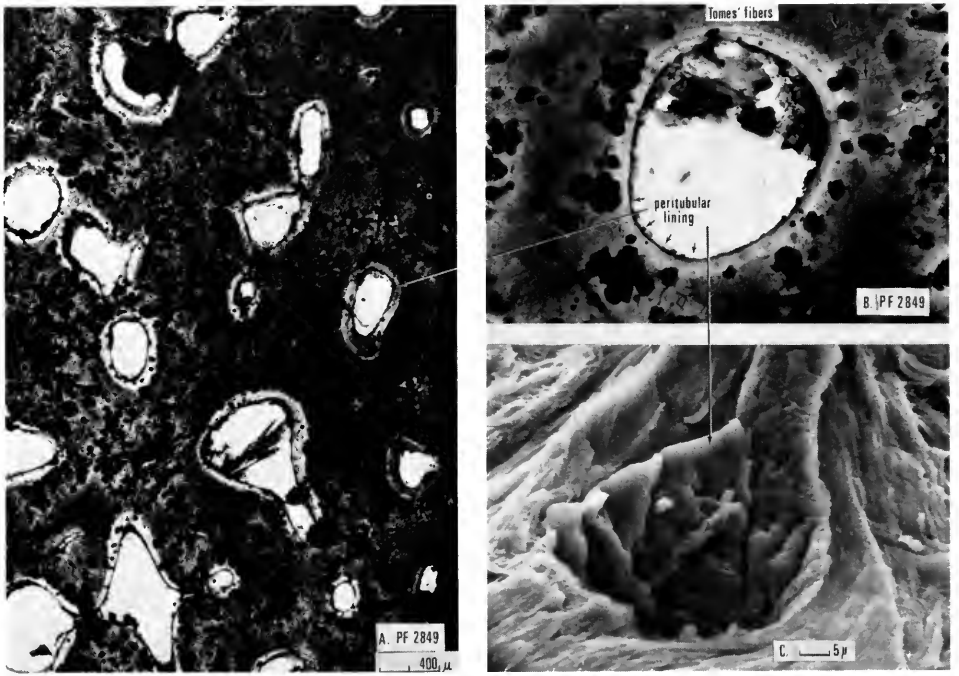


PLATE 5. **a**, PF 2849, Brightfield view of a coronal section through Type 2 trabecular dentine demonstrating the abundance of peritubular tissue. **b**, PF 2849, Enlargement of a single denteon demonstrates Tome's fibers and peritubular lining. Note the internal margin of the peritubular tissue that has eroded away the odontoblasts. **c**, Scanning micrograph of recent Type 2 trabecular dentine from the hammerhead shark shows surface characteristics of the peritubular lining.

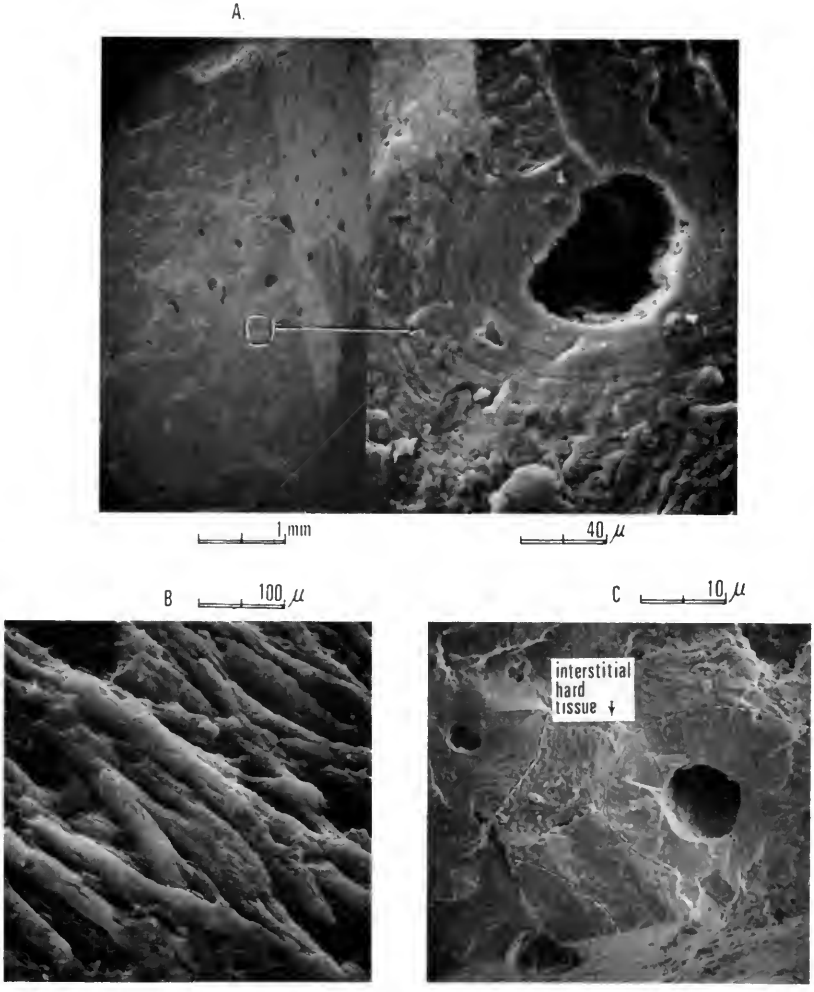


PLATE 6. PF 2849, **a**, Fiber-mineral bundles within the denteon wall are arranged in circular fashion. **b**, Scan at 2,000 magnifications of the denteon wall shows branching fiber bundles. **c**, The fracture pattern of the interdenteonal tissue is that of a woven-fibered hard tissue. The interstitium between denteons seen here is characteristic of Type 2 trabecular dentine.

duced and frequently absent. Type 2 is immediately subjacent to Type 1 and constitutes the central crown region and most of the tooth base. The denteons are separated by an acellular interdenteonal hard tissue (pl. 3a). The type of interstitium found here has been referred to by Radinsky (1961) as interosteonal hard substance and Peyer (1968) as cell-free interosteonal hard substance. The

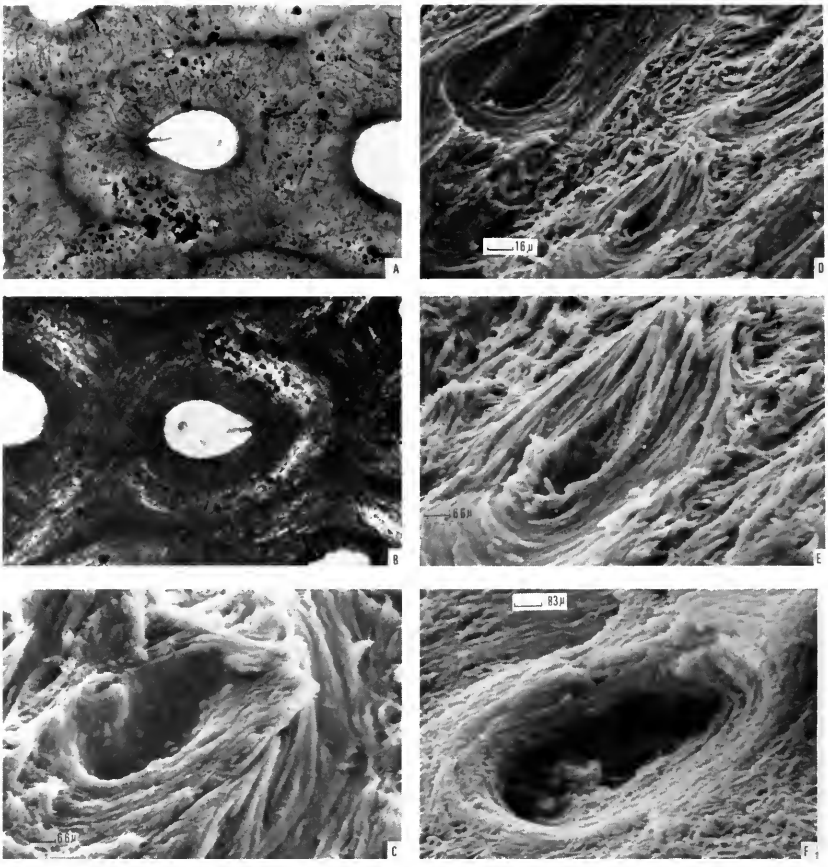


PLATE 7. **a**, Fossil denteon in Type 2 trabecular dentine in brightfield shows Tome's fibers quite clearly. The black dots are radio-opaque pyrite. **b**, In polarizing light the denteon is seen to be composed of an inner dark ring of different refraction and therefore different fiber-crystal orientation than the bright outer ring. **c**, Recent Type 2 trabecular dentine from the hammerhead shark, shows a consistent inner ring of transversely oriented fibers, and an outer ring of more longitudinally oriented fibers. **d, e**, In modern tissue as in the fossil, denteons with lamellae of common fiber orientation are interspersed with lamellae of alternating pitch. The interdenteonal hard tissue is the frothy material between the denteons. **f**, A natural growth surface of a denteon from a modern Isurid shark shows the rope-like substructure of the denteon wall.

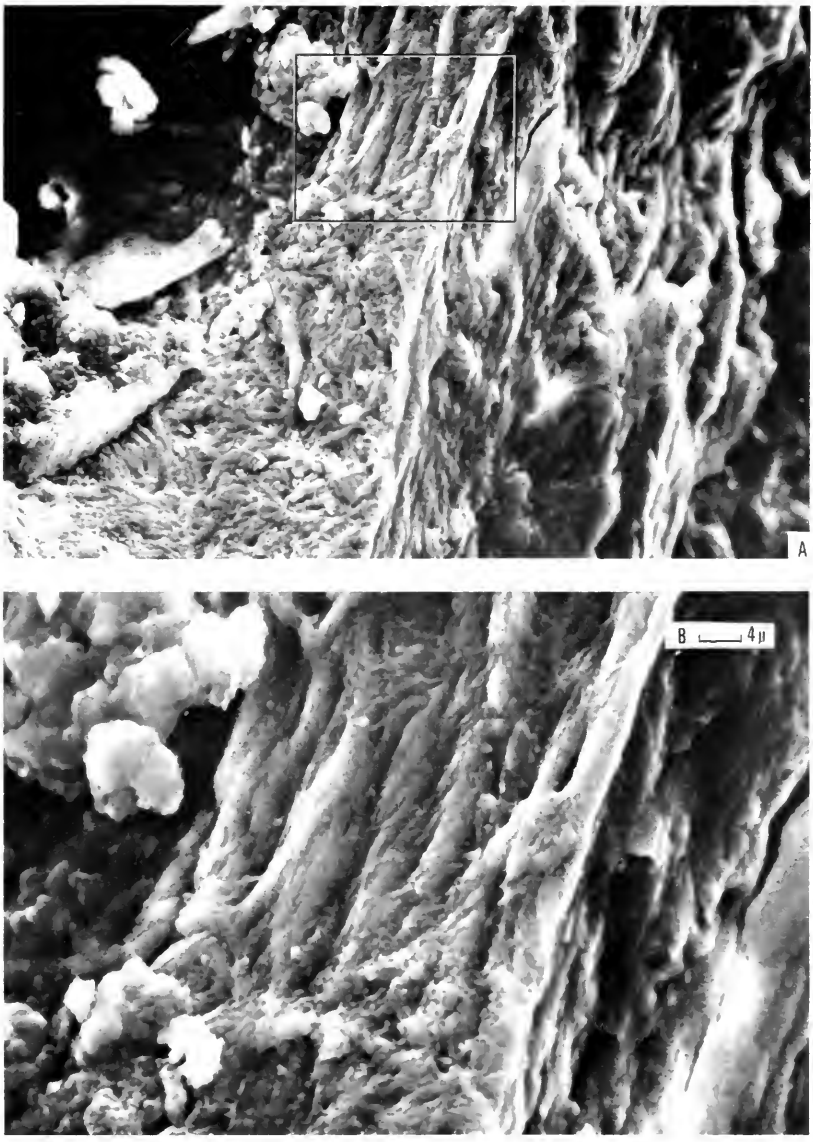
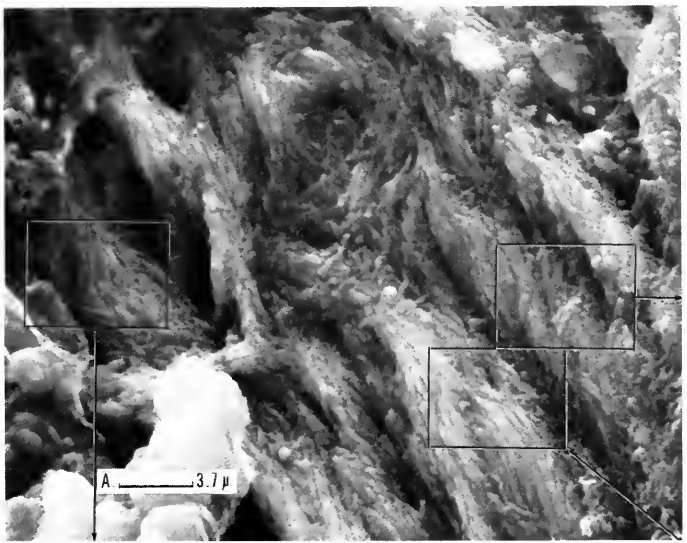


PLATE 8. **a**, The denteon wall appears to be composed of continuous and discontinuous super-bundles in left-handed coils, PF 2848. **b**, Lamellae arranged circularly around the lumen are composed of spiralling left-handed super-bundles.



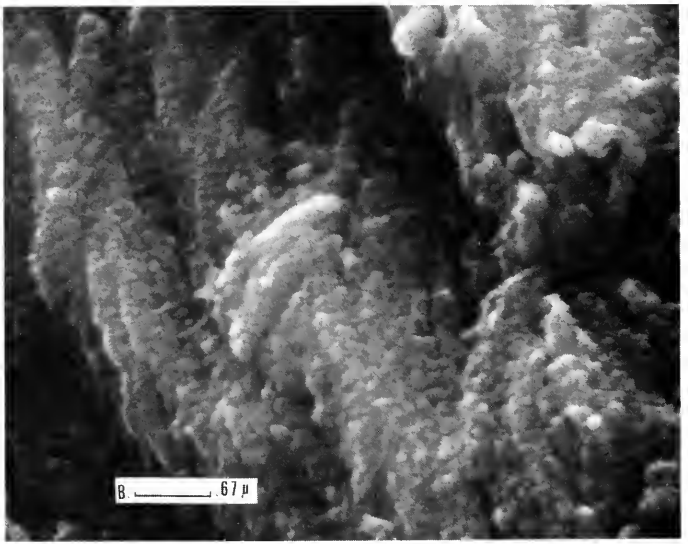
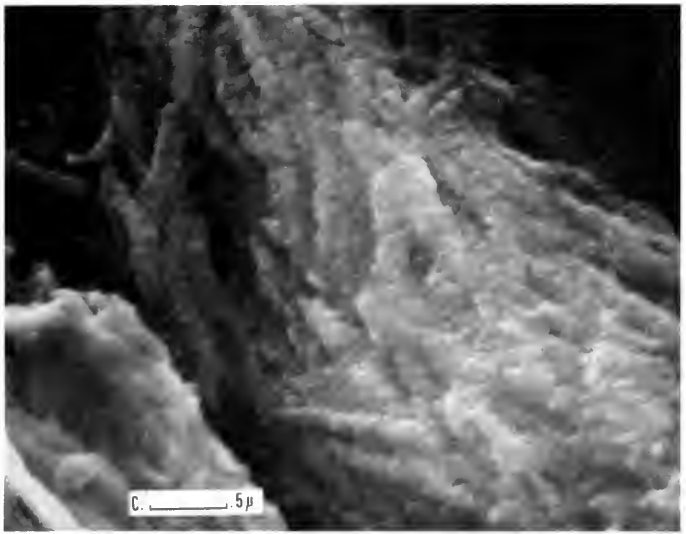


PLATE 9. **a**, Higher magnification of the field shown in Plate 8 of PF 2848, **b**, Structure of a single lamella of the denteon wall is composed of left-handed super-bundles. Two can be seen clearly on the surface of this lamella.



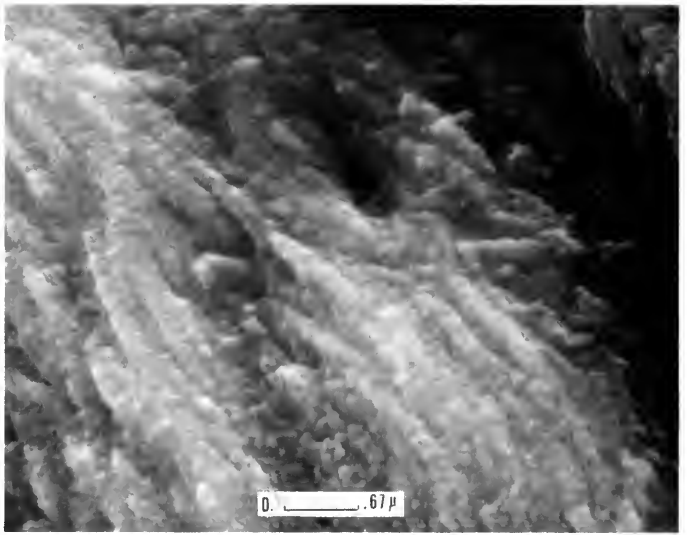


PLATE 9. **c**, This section of a lamella is not organized into clearly delineated super-bundles, but is composed of branching fiber-crystals. The fibers appear to be complexed with a mineral, probably fluorapatite and a bituminized carbohydrate phase. **d**, Longitudinal fiber arrangement is discontinuous here. The fibers are broken off distally and would continue upward and away from the viewer. The upward pitch of these fibers can only be approximated at about 15-20 degrees.

tissue is here called interdenteonal because it is the interstitium of denteons and is laid down by odontoblasts. A very distinct fracture pattern was observed on untreated natural surfaces of this tissue, as shown in Plate 6c. The fracture pattern of this interdenteonal tissue is that of a woven-fibered hard tissue. The denteons in Type 2 characteristically have a thick peritubular lining (pl. 5a, b, c). The substance is quite distinct from the interdenteonal tissue under polarizing light; it has a different birefringence and does not have the fracture pattern of a woven fibered tissue (pl. 7b). The margin between the peritubular lining and the innermost lamella is regular and the absence of odontoblast spaces suggests an erosion surface instead of an initial growth border (pl. 5a, b). The substance is probably a lime salt, phosphate accumulation — a mineral storage to which the circulatory system has ready access. The interdenteonal tissue is avascular and therefore cannot serve as a ready access mineral store in the adult tooth as can the peritubular lining. Type 3 trabecular dentine is found in the outer portion of the tooth base, the external millimeter of all of the non-crown dentine (pl. 3a). It is a spongy system of open trabecles lacking denteon organization. The spongiosum grades into Type 2 in the internal portion of the root.

It is the ultrastructure of Types 1 and 2 that scanning has significantly added to — that is the nature of the denteon and its matrix. Scanning evidence emphasizes the similarities in extracellular events between dentine and bone. It is well known that the denteon analog, the osteon in secondary bone, grows by the internal apposition of successive lamellar rings, but the ultrastructure has not been previously demonstrated for dentine. Annular concentric growth within a lamella of fossil trabecular dentine appears to be accomplished by left-handed coils of super-bundles (pl. 8a, b). This growth arrangement is confirmed in modern material. A scanning micrograph of a growing denteon in an immature *Isurid* tooth shows clearly the rope-like spirals within the lamella which when mature will enclose the denteon lumen (pl. 7f). The constituents of the rope-like bundles are organized at the scanning level like a structural protein complexed with a mineral and a bituminized carbohydrate phase. The fibrous bundles appear to have an exoskeleton probably of fluor-apatite, which coalesces each to adjacent bundles in a fashion exactly like that described for bone (pl. 9a-d). Denteons, because they do not remodel, retain their original structure, a structure in which there is evidence of continuous and discontinuous spirals of super-bundles. The spatial relationship between apatite crystal distribution and structural protein is similar to that found in bone.

There is alternation of pitch in the lamellar spirals of the denteon wall. In polarizing light the fossil denteon is shown to have bands of different refractive indices indicating a difference in fiber-crystal orientation. The bright ring seen in Plate 7a is a lamella of longitudinally oriented fibers and crystals. The dark inner band is of transversely oriented constituents. Denteons with alternate pitch lamellae are equally distributed in Types 1 and 2 trabecular dentine. The alternate pitch and common fiber orientation is seen quite clearly in modern trabecular dentine from a hammerhead shark (pl. 7c, d, e). The lamellar organization of trabecular dentine seen here agrees with that found in bone.

Enameloid

The thin outer crown covering of enameloid at the light microscope level is best seen in Plates 2 and 3. Enameloid is considered a hyper-mineralized type of dentine formed inside the basement membrane of the enamel epithelium and therefore not the homolog of enamel in higher vertebrates, which is of ectodermal origin. Peyer (1968) distinguishes the enameloid of elasmobranchs from the true enamel of reptiles and mammals on the basis of its (1) not originating by the mineralization of cell processes of ameloblasts, (2) by lacking the birefringence of true enamel, and (3) the direction of growth being not centrifugal but centripetal. Enameloid is, in fact, formed inductively by ectodermal and mesodermal elements (Shellis and Miles, 1974). The enameloid seen here is essentially identical to that found in other elasmobranch teeth.

Discussion

Trabecular dentine, on the whole, has attained certain physiological features that are significant in the evolution of hard tissues in general. Paleozoic trabecular dentine behaves in every way like the modern tissue at the histologic and ultrastructural levels. Bone Haversian systems share several homologous properties with the denteon in trabecular dentine. The denteon has acquired the centripetal growth pattern that proceeds by the successive apposition of lamellae to surround an arteriole and venule. The presumed collagen fibrils in the denteons are roughly parallel as they are in Haversian bone. The inotropic calcification system is the same in both. Angiogenesis and microcirculation determine the structure of Haversian bone and this is the inference made here for trabecular dentine: that it is formed as a result of the vascular invasion of the anlagen by capillaries. There is a spatial relationship between fluor-

apatite crystal distribution and collagen periodicity as there is in bone.

One of the major points of difference of trabecular dentine from bone is that there is no clastic resorption. It would serve no functional advantage in a system of continually replaced teeth. The only remodelling phenomenon seen is the perivascular erosion and infilling, for which there is only indirect evidence. An additional difference is that the denteons in trabecular dentine do not incarcerate blast cells into lacunae as bone does.

This is not to suggest that trabecular dentine is the precursor of bone. The advent of bone, however, was by transformation within an existing developmental system and trabecular dentine demonstrates features of a system fundamental to many hard tissues. It is not clear what parameters of growth and function evolved in the precursor of these hard tissues and which elements constitute the final modification.

EMBRYOLOGY

Introduction

It is now a relatively secure developmental fact that structures originating from epithelia require a mesenchymal association for both development and differentiation into adult forms (Fleischmajer and Billingham, 1968). This fact is well illustrated in the studies of the kidney (Grobstein, 1955), the integument and its appendages (Wessells, 1967, 1970; Kollar and Baird, 1970a, b), the lungs (Spooner and Wessells, 1970; Wessells, 1970), pancreas (Dietelein-Lièvre, 1970), and some portions of the thymic immune system (Harrison et al., 1970), and many others. Involvement in such a diversity of systems speaks toward the epithelial-mesenchymal interaction as being a fundamental developmental process in the ontogeny of present day organisms. It is not unreasonable, therefore, to attempt to explain certain observations of developmental events in fossil forms by invoking similar processes.

Edestus heinrichii, unrelated to the modern radiation of sharks, represents an evolutionary endpoint. To date there has been no satisfactory explanation of the ontogeny of the diverse types of hard tissue found in this organism, especially in the case of trabecular dentine.

Current hypotheses (Ørvig, 1967c) relating to the origins of type 2 trabecular dentine center around the idea that the presumptive

odontoblasts differentiate into competent dentine-secreting cells retain this competency at the external borders of the developing tooth to produce type 1 trabecular dentine, but somehow lose this competency in the interior portions, dedifferentiate, and then proceed along an alternative developmental program to become osteoblasts involved in the deposition of the interstitial bands (if, in fact, they are bone), then redifferentiate again into odontoblasts which secrete the trabecular denteons. This hypothesis is untenable from the standpoint of a single cell population undergoing three developmental sequences. An explanation based on observations of the embryology of modern organisms is more economic. Indeed, Herold (1971) has shown that one cell type undergoing maturation, but not re-differentiation, produces the pattern of osteodentine seen in certain teleost teeth.

Hypothetical embryogenesis of trabecular dentine in E. heinrichii

The participation of the mesenchyme in the induction of tooth ontogeny was clarified by Wild (1955a, b) with his observation that the mesenchymal component of urodele teeth migrated from their origin in the neural crest. Since that time there has been considerable controversy over the major determinant of tooth development, i.e., whether the mesenchyme or the oral epithelium determines the ultimate type of dentition at a particular locus. Pourtois (1964) and Miller (1969) have shown that in the stages preceding epithelial invagination, the isolated epithelial components can develop into the correct dental type (incisor or molar). However, Kollar and Baird (1970a, b) demonstrated the inductive predominance of the dental papilla in later stages of tooth morphogenesis. These contrary results can be easily reconciled if one were to propose that initially the epithelium is provisionally determined and exerts an inductive effect on the presumptive dental mesenchyme which, as tooth development proceeds, assumes complete control over the final morphogenetic outcome. The implication here, then, is that the cells of the dental papilla become autonomous in their ability to differentiate after their initial induction by the epithelial component. Huggins et al. (1934) demonstrated this fact in developing dog teeth by implanting isolated mesenchymal components into abdominal muscles, where calcified dentine was formed by the odontoblasts in the absence of direct epithelial contact with the enamel organ. The nature of the dentine formed was varied, from orthodentine grading through trabecular forms to material indistinguishable from true bone. This latter finding is of utmost interest in the discussions of

the origins of the trabecular dentine found in the teeth of fossil sharks and indeed of the modern elasmobranchs with similar hard tissue types. The major consideration here is that the trabecular pattern arose when the odontoblastic layer was in the presence of, but not in contact with, the internal enamel epithelium. This suggests that the resultant interaction was incomplete in the sense that there was no physical epithelial-mesenchymal interface to order the secretory events. It is also significant that in these experiments there seemed to be a time dependency for the pattern of dentine seen, with the trabecular dentine formed in the implants continued beyond 24 days. Combining these two points, one has the basis for an interesting speculation for the genesis of a trabecular dentine pattern: trabecular dentine is the natural maturation pattern of secretion by induced odontoblasts in the absence of an internal enamel epithelial interface, but in the presence of a distant, indirect or primitive epithelial influence.

Against the above background it is possible to construct a sound hypothesis regarding the generation of trabecular dentine during the morphogenesis of elasmobranch teeth. Peyer (1968) describes the histiogenesis of the teeth of two modern sharks, *Squalus acanthias* and *Scylorhinus canicula*, and shows that, in spite of an evaginating surface development of the teeth, all embryological cell types characteristic of higher vertebrate forms are present. Here we shall term the analog of the internal enamel epithelium, the internal odontogenic epithelium, to avoid assumptions about the developmental future of this tissue.

Peyer's microscopic sections show that during early morphogenesis there are presumptive odontoblasts subjacent to the internal odontogenic epithelium and thus subjected to the initial inductive influence. As maturation of the tooth anlagen continues, many of the initially induced cells become crowded into the center of the dental papilla, creating a population of potentially dentine-secreting cells in the deep interior of the tooth. It is reasonable to propose, as the observations of Huggins et al. (1934, 1970) suggest, that this cell population, deprived of an epithelial-mesenchymal interface with which to orient its secretory activity, will nevertheless retain its secretory ability long after exposure to the epithelial inductive influence and deposit dentine circumferentially to produce the trabecular pattern. Those odontoblasts remaining juxtaposed to the internal odontogenic epithelium align their secretion product along the epithelial-mesenchymal interface. This product is reminiscent of

orthodentine but in *E. heinrichii* would actually be enameloid, produced under the influence of the ontogenetically primitive epithelium. The epithelial contribution could be either a purely inductive effect on the subjacent mesenchyme or a product manufactured by the epithelium and incorporated into the enameloid with the mesenchymal component. It is noteworthy in this regard that Shellis and Miles (1974) have demonstrated a matrix component in the enameloid of certain teleost fishes that is secreted by the internal dental epithelium. Indeed, it is well known that matrix materials play an essential role in the differentiative program of tissues invoking epithelial-mesenchymal inter-actions (Dodson, 1963; Fell and Grobstein, 1968; Wessell and Evans, 1968; Bernfield, 1970; Goetnick and Sekellick, 1972; Vracko and Benditt, 1972). Thus, while the exact timing of developmental stages, and the functional life span of the cells involved in trabecular dentine deposition are not known, it is tempting to look toward such matrix influences as an organizer for the succession of developmental events.

The more centrally located odontoblasts could be subject to an inductive gradient from the internal odontogenic epithelium to produce the types 1 and 2 trabecular dentines seen in *E. heinrichii.* It is germane to note that in several modern radiations of sharks, the appearance of a type 2 trabecular dentine occurs concomitantly with the disappearance of the internal odontogenic epithelium (Peyer, 1968), at which time the autonomy of the most centrally placed odontoblasts might be assured.

While the trabecular morphology seen in *E. heinrichii* might be explained by invoking the above embryological processes, the presence of the interstitial banding pattern seen is thus far not treated by these hypotheses. However, the ideas presented so far can easily include such a phenomenon. Considering the histology of the tooth primordium before hard tissue deposition, one should recall that the mesenchyme of the dental papilla is composed of two populations of cells — the neural crest cells, which are the presumptive odontoblasts, and a population of uncommitted pluripotential mesenchymal cells which will eventually differentiate into such diverse tissues as fibroblastic connective tissue, vascular elements, and other mesodermally derived tissue. If, in the course of deposition of the dentine by the odontoblasts located in the central portions of the tooth, some of the pluripotential mesenchymal cells are trapped between the expanding trabecles of dentine, one could easily theorize the establishment of an interdenteonal fibroblast population.

The findings of Huggins et al. (1934, 1970) showed that implants of both odontoblast and matrix components can induce bone and cementum formation from surrounding fibroblasts, one can envision a similar process occurring with these trapped fibroblasts. That is to say, the expanding dentine network might exert an inductive effect on the interdenteonal fibroblasts to produce hard tissue metaplasia manifested as the interstitial bands. The absence of banding in the type 1 dentine seen in *E. heinrichii* could be accounted for by the exclusion of fibroblasts from the region immediately subjacent to the internal odontogenic epithelium, this area being exclusively populated by the neural crest presumptive odontoblasts.

Evidence for such a developmental program is present in both the light and the scanning electron micrographs discussed in the first section of this paper. From the light micrographs it is evident that the interstitial bands are acellular. However, the scanning electron micrographs show that the matrix of the interstitial bands is composed of woven fibers, probably originally collagenous, oriented perpendicular to the axis of the dentinal matrix. This suggests that two different cell populations separately secreted their respective matrices, and that the interstitial population oriented its product along the lines of stress between adjacent denteons. The interstitial hard substance may be related to cementum, judging from the loosely woven texture of the fibrous matrix, the lack of incarcerated cells usually associated with true bone, and the interrelationship with the forming dentine. It is noteworthy that the banding seen in *E. heinrichii* is not the type discussed by Ørvig (1967c) under the heading of osteodentine or osteo-semi-dentine, where a definite bony superstructure precedes the deposition of dentine. The evidence in the sections of *E. heinrichii* indicates that the dentine was the first of the hard tissues to be laid down, since the interstitial bands are discontinuous and narrow, suggesting that indeed they are the result of isolation of fibroblastic strands at the matrix border of the enlarging trabecles. This last point must be stressed, since primary bone formation with secondary dentine deposition should be manifested by well-defined, continuous interstitial bands. The existence of the hard tissue class known as mesodentine is attributed to the presence of a mesenchymal cell population intermediate between osteoblasts and odontoblasts (Ørvig, 1967c). This point is not acceptable when one considers the mass of data showing that in a broad spectrum of present day organisms possessing denticles, the odontoblasts do not originate from the same precursor population as do the osteoblasts (Horstadius, 1950; Wild, 1955a; Fowler, 1972;

Kelly and Bluemink, 1974). Odontoblasts derive from the neural crest and are thus mesectodermal, whereas osteoblasts derive from a purely mesodermal cell population. Mesodentine can be more logically considered within the above concept, originating via trapping of fibroblastic elements within the expanding dentinal matrix, rather than as a result of programmed loss of odontoblast function in the course of the secretory process.

In further pursuit of the question of the presence of bone in *E. heinrichii*, one must consider whether the trabecles themselves represent a stage in the evolution of bone. There are several developmental dissimilarities that preclude making such an association. First is the difference in the cellular origins already discussed at length above. Second, previous discussions propose that the dentinal trabecles of *E. heinrichii* are formed in a centripetal direction away from the presumptive regions of the interstitial bands, and toward elements of the concurrently developing vascular supply, i.e., toward the nutritional supply of the odontoblasts ahead of the dentine and predentine deposition which is modulated by an expanding vascular network. The smaller dentine pattern seen in the type 1 dentine could well be the result of such a process occurring around abundant terminal small vessels near the periphery of the tooth. Third, there is no evidence in any of the material from *E. heinrichii* studied of hard tissue clasis along the free edge of the denteons, as shown by the absence of irregular erosions, thus eliminating an important developmental aspect of most bone, since an elementary feature of more rapid bone growth and development is the role of osteoclastic resorption in the modelling process (Gruneberg, 1937). However, it is generally agreed that both intramembranous and endochondral bone ontogeny in higher vertebrates proceeds with osteoblastic activity preceding the osteoclastic contribution to remodelling.

The presence of trabecular dentine in edestids might thus represent a level in evolutionary advance to modern bone with complete dependence on blastic activity for this process. Clastic activity might then be interpreted as an evolutionary adaptation to adjust final form more adequately to ultimate function. However, the relation between bone and edestid trabecular dentine cannot be established on this basis alone.

Finally, the Tome's processes within the dentinal matrix function differently from bone canaliculi, since their primary purpose is the mineralization of the matrix, not the nutritional supply of the embedded cell population, as is the case for the canaliculi. Herold

(1971) concludes that teleost osteodentine bears no relationship to bone in that there are critical differences in the formation process, matrix structure, and in cellular components. However, unlike the teleost system studied, the edestid trabecular dentine does show evidence of appositional growth within the denteons, a feature used by Herold (1971) to disassociate osteodentine from bone.

In conclusion, what has been presented in this section is an attempt to explain the presence of the various hard tissues seen in *E. heinrichii* on the basis of what is known about the histiogenesis of similar tissues in modern vertebrates. It was not intended to place this edestid in an evolutionary perspective, nor to relate it to other forms with similar or dissimilar histology. Rather, the purpose here was to explore an approach to the interpretation of paleohistology with the suggestion that application of such a perspective to similar material might lead to a more rational construction of evolutionary history.

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