Studies on the Developing Enameloid of a Fish (*Hoplognathus fasciatus*)

I. Mineralization Pattern of Enameloid Matrix

by

Sohiti ISOKAWA,* Minoru TSUBOUCHI,* Keisuke AOKI,* Mitsuo IMAI,*
Atsushi KAWAI* and Sakae TSUCHIDA*

Introduction

There are two principally different opinions about the mineralization of the human enamel matrix. DIAMOND and WEINMANN[1] observed that the formation of the enamel matrix was parallel to the incremental lines of RETZIUS and the matrix was insoluble in acid. After the enamel matrix was formed completely, the secondary calcification, which they called maturation, commenced. This extended from the incisal edge or the tip of the cusp toward the cervix almost perpendicular to the long axis of the tooth.

On the other hand, ENGFELDT and HAMMARLUND-ESSLER[2], ALLAN[3, 4], CRABB and DARLING[5, 6, 7] recently studied the mineralization of the human enamel by means of X-ray absorption. They concluded that the enamel mineralization was observed shortly after the commencement of organic enamel matrix deposition. They found no evidence that the mineralization started at the enamel surface, that is, at the incisal edge or the tip of the cusp and spread at right angles to the tooth long axis.

At the present time, there is no study of pattern of mineralization of fish tooth enameloid (enamel). In the present study, the authors concerned themselves with the mineralization pattern of the fish tooth enameloid of *Hoplognathus fasciatus*.

Material and Methods

The upper and lower jaws of *Hoplognathus fasciatus* were used for this study. The number and arrangement of the teeth of this fish are different in the young and the adult forms. The adult fish have beak-like jaws consisting of the teeth and their surrounding bones (Fig. 1). The successional teeth consisting of about ten different stages of formation from an initial tooth germ to a prefunctioning tooth are contained in the beak-like jaws. The functioning teeth are ankylosed with bones.

The jaws including teeth and tooth germs were fixed in 10% neutral formalin and dehydrated with alcohol. After the jaws were embedded in a plastic material, they were sectioned longitudinally (showed with arrows in Fig. 1) 40–120 microns thick with a Gilling Bronwill thin sectioning machine. Historadiographs of these sec-

* 磯川宗七, 坪内実, 青木啓祐, 今井三男, 河合温, 土田栄, *Dept. of Anatomy, Nihon Univ. School of Dentistry, Tokyo, Japan (Director: Professor Sohiti Isokawa)*

tions were made with a Softex CMR type X-ray machine. Both the historadiographs and the ground sections were compared with a microscope.

Fig. 1 Beak-like upper jaw of *Hoplognathus fasciatus*, A: frontal aspect, B: lateral aspect, arrows show sectioning direction for Fig. 2, $\times 3$.

Observations

As mentioned above, a complete series of developing teeth was observable within a section of a fish jaw. A negative picture of the ground section of the jaw and its historadiograph are shown in Fig. 2A and B in which one can see the teeth in their different formative stages. Almost all of the teeth show that the enameloid and mantle dentin areas appear black, indicating a high degree of X-ray absorption in the historadiograph (Fig. 2B). However, the circumpulpal dentin of the teeth and surrounding bones appear grey indicating a low X-ray absorption. Some of the enameloid of the immature teeth in the deep part of the jaw show a low or almost negligible X-ray

Fig. 2-A

Fig. 2-B

A negative photomicrograph (A) and its historadiograph (B) of a ground section of a jaw, arrows show early developing teeth, unstained, $\times 6$.

Fig. 3 A photomicrograph (A) and its autoradiograph (B) of an immature enameloid. E: enameloid, P: dental papilla, unstain, $\times 60$.

Fig. 4 Autoradiograph of three immature enameloid, $\times 24$.

Fig. 5 An illustration of the mineralization pattern of the fish enameloid.

absorption. This denotes that, in these immature teeth, the mineralization of the enameloid matrix is still incomplete. The outlines of the completed enameloid matrix can be seen in the ground section (Fig. 2A). This demonstrated that an examination of the spreading of the radiopaque zone in the immature enameloid by means of X-ray absorption methods is an excellent way to investigate the mineralization pattern of the forming enameloid.

An examination of the enameloid matrix of three teeth, shown by arrows at the bottom of Figure 2, indicates that the X-ray absorption does not extend uniformly through the entire enameloid thickness. There are differences in a radiopacity of the enameloid in each of the three teeth. A study of these differences in radiopacities, however, explains the mineralization pattern of the enameloid.

Fig. 3A is a 40-micron thick ground section of a tooth. The thickness of the enameloid matrix is equal to that in the matured tooth. However, there is as yet no evidence of dentinogenesis along the outer surface of the dental papilla. From this, it is implied that the development of the dentin starts in this fish tooth after the future outline of the organic substance of the enameloid is completed.

In the photomicrograph (Fig. 3B) which is the historadiograph of Fig. 3A, one can see a high radiopaque band along the dentinoenameloid junction and also a narrow X-ray opaque band along the enameloid surface. Moreover, in the tip area of the enameloid there is a radiopaque cord connecting the mineralized band of the enameloid surface and that along the dentinoenameloid junction.

Fig. 4 is a historadiograph of three young teeth. In the tooth on the left, only the enameloid shows a radiopaque band along the dentinoenameloid junction. The

enameloid of the middle tooth has a thicker mineralized band than that of the tooth on the left. This enameloid also shows a beginning radiopaque band on its surface. As shown in Fig. 3B, one can see a radiopaque cord which binds the X-ray absorption bands of the enameloid surface and that of the dentinoenameloid junction. In the tooth on the right, the spreading of the mineralization extends to the middle layer of the enameloid. The dentin development has already started and shows a low level of X-ray absorption. The mineralization of the middle layer of the enameloid begins later.

The mineralization pattern of the enameloid of the teeth of this fish is illustrated in the diagrams in Fig. 5. The spreading of the mineralization is shown in black in the illustration. Fig. 5 suggests that the mineralization of this tooth first appears in the enameloid along the future dentinoenameloid junction. This is followed by mineralization of the enameloid surface. A radiopaque cord then binds the X-ray absorption bands of both sides of the enameloid. It is fairly late before the mineralization is completed in the middle layer of the enameloid.

Discussion

No one knows whether or not the study of DIAMOND and WEINMAMM[1] on the mineralization pattern of human enamel is correct and also whether or not the reports of ENGFELDT and HAMMARLUND-ESSLER[2], ALLAN[3 and 4], CRABB and DARLING[5, 6, 7] etc. are true. According to this study, however, the enameloid of this fish has a peculiar mineralization pattern. It is apparently different from the patterns of calcification of the human enamel mentioned above. It is believed that the enameloid mineralization of this fish started along the dentinoenameloid junction and the enameloid surface. The historadiographs of the thinner enameloid ground section (40–50 μ thick) seemed to us to explain very clearly this mineralization pattern. SUGA[8] suggested that the mineralization pattern of guinea pig enamel is similar to that of the fish enameloid in this study. The mineralization pattern of the enamel in various animals should be more thoroughly studied.

It is also believed by some people that fish tooth, unlike human tooth enamel, is not necessarily formed from an ectodermal tissue. In 1950 KVAM[9] reported a fish tooth enamel of mesodermal origin. YOSHITANI[10] also reported sea-bream tooth enamel originated from both the mesoderm and ectoderm. ISOKAWA[11] wrote in his paper in 1955 that dentinogenesis might start after the outline of the future enameloid had been formed by its organic matrix. This assumption was confirmed by this study. These facts may have a close relationship with the developmental origin of the enamel.

Conclusion

The mineralization pattern of the enameloid of *Hoplognathus fasciatus* was examined using historadiographs and ground sections.

It was suggested that the mineralization of this fish tooth first started in the enameloid along the future dentinoenameloid junction and following this mineralization next appeared along the enamel surface. A radiopaque cord of mineralized enamel then bound together two radiopaque bands on both sides of the enameloid. The middle layer of the enameloid then became mineralized later by the spreading of the radiopaque bands from two mineralized bands of the enameloid.

It was also demonstrated that the mineralization of the enameloid commenced after the future outline of the matrix was completely formed.

(The authors wish to acknowledge their thanks to Dr. G. VAN HUYSSEN, Indiana University School of Dentistry, for his useful suggestions)

References

- [1] DIAMOND, M. and WEINMANN, J. P.: The enamel of human teeth, Columbia Univ. Press, New York, (1940).
- [2] ENGFELDT, B. and HAMMARLUND-ESSLER, E.: Studies on mineralized dental tissues. IX. A microradiographic study of the mineralization of developing enamel, *Acta odont. Scand.*, **14**, 271-289, (1956).
- [3] ALLAN, J. H.: Mineralization of human enamel, *Nature*, **180**, 1362, (1957).
- [4] ALLAN, J. H.: Investigations into the mineralization pattern of human dental enamel. Part II. X-ray absorption studies, *J. dent. Res.*, **38**: 1108-1118, (1959).
- [5] CRABB, H. S. A. and DARLING, A. I.: The pattern of mineralization of human dental enamel, *Proc. Roy. Soc. Med.*, **52**, 118-122, (1959).
- [6] CRABB, H. S. A. and DARLING, A. I.: The gradient of mineralization in developing enamel, *Archs oral Biol.*, **2**, 308-318, (1960).
- [7] CRABB, H. S. A. and DARLING, A. I.: The pattern of progressive mineralization in human dental enamel, Pergamon Press, Oxford, (1962).
- [8] SUGA, S.: Personal communication (1966).
- [9] KVAM, T.: The development of mesodermal enamel of piscine teeth, *Det Kgl. Norske Vidensk. Selsk. Trondheim*, (1950).
- [10] YOSHITANI, M.: Histogenetic studies on the teeth of *Pagrosomus unicolor*, a genus of sea bream, *J. Japan Stomat. Soc.*, **26**, 811-832, (1959), (Japanese).
- [11] ISOKAWA, S.: Morphological studies on teeth of fishes. IV. Teeth of file-fishes, *Zoolog. Mag.*, **64**, 194-197, (1955), (Japanese).