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# Fluoride Concentration in Teeth of Tetraodontiform Fishes and Its Phylogenetic Significance

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**Abstract** Quantitative fluoride analysis by electron microprobe was performed on the teeth and bone of marine teleosts of the order Tetraodontiformes (26 species). This order contains two suborders, namely, the Balistoidei which is a relatively primitive group and the Tetraodontoidei which is more advanced. Fluoride concentration in the enameloid of all the fishes examined from the Balistoidei was higher than 2.31%, whereas that in the enameloid of all the tetraodontoids except for the Molidae was lower than 0.22%. The enameloid of fishes of the Acanthuridae of the order Perciformes, from which the Tetraodontiformes may have derived, contained fluoride higher than 3.85%. The dentin and bone of all the fishes examined contained flouride lower than 0.82% and 0.57%. These results seem to indicate that the fluoride concentration in the enameloid is closely related to the phylogeny of fishes and that fishes of the Balistoidei have a peculiar ability to concentrate the fluoride to the enameloid (probably in the ectodermal enameloid-forming cells) and those of the Tetraodontoidei have lost this ability in the course of evolution.

Fish teeth consist of enameloid and dentin. The enameloid is analogous to the enamel of mammalian teeth and is similarly highly mineralized. The organic matrix of enameloid comprises both ectodermal and mesodermal products, while that of enamel contains only ectodermal products. It is known that shark's enameloid is composed of fluorapatite with the fluoride content as high as about 3.5% (Trautz et al., 1952; Glas, 1962; Büttner, 1966; Suga et al., 1978; Daculsi and Kerebel, 1980), whereas mammalian enamel is made of hydroxyapatite with a very low fluoride level (about 0.05～0.2%) (Brudevold and Söremark, 1967).

Our previous quantitative electron microprobe analysis of the teeth of various teleost and elasmobranch fishes indicated that fishes may be classified into two groups in terms of the fluoride content in the enameloid: one group containing fluoride of more than about 2.50% in the enameloid; and the other containing less than about 0.17%. The fluoride concentration in the enameloid appears to be related to fish phylogeny rather than the fluoride concentration in the environmental water (Suga et al., 1977, 1978, 1979, 1980). This is contrary to the general concept that the fluoride concentration in the enamel is closely related to that in drinking water (Brudevold and Söremark, 1967).

In order to investigate further the relationships among the fluoride concentration in the enameloid, the fish species and the environmental water, quantitative electron microprobe analysis was carried out on the teeth of many marine forms of the Tetraodontiformes collected systematically. Preliminary results were reported earlier (Suga et al., 1978).

The Tetraodontiformes are considered to have evolved from the Perciformes during the Eocene period of the Cenozoic Era (Tyler, 1968; Winterbottom, 1974; Matsuura, 1979; Tyler, 1980). Living examples are classified into two suborders, the primitive Balistoidei which contains 6 families and the more advanced Tetraodontoidei containing 4 families (Tyler, 1968, 1980).

## Materials and methods

The specimens used for the present study comprised 13 species of 6 families of the Balistoidei and 13 species of 4 families of the Tetraodontoidei (Table 1, Fig. 4). Specimens were obtained mainly from the Japanese coast and the East China Sea. Some species (i.e. *Chilomycterus affinis*) were also obtained from the Malaysian coast. For comparison, the teeth of two perciform specice (*Acanthurus dussumieri* and *A. nigricans*) belonging to the Acanthuridae (Table 2), from which the Tetraodontiformes may have derived (Breder and Clark, 1947), and unerupted human enamel and dentin were also examined. They were stored in isopropyl alcohol and/or 10% formalin.

The erupted and developing teeth and their surrounding jaw bone were embedded in polyester resin after dehydration in an ascending series of concentrations of alcohol. Ground sections about 60~70 μm thick were made with a grindstone after sectioning with a diamond saw.

The ground sections were first microradiographed using soft X-ray apparatus in order to see the mineralization pattern and then mounted on a polished aluminum block with epoxy resin. After applying a thin coating of carbon to the polished surface of specimens by evaporation in a vacuum to form a conducting layer, electron microprobe quantitative and line scan analyses were performed.

The electron microprobe apparatus used for the present study was Shimadzu-ARL, type

Table 1. Locality and number of specimens from the order Tetraodontiformes.

| Species | Locality | Number |
|---|---|---|
| Balistoidei | | |
| Triacanthodidae | | |
| *Triacanthodes anomalus* | Kohchi-ken, Japan | 2 |
| Triacanthidae | | |
| *Triacanthus brevirosteris* | Daiohzaki, Mie-ken, Japan | 1 |
| Balistidae | | |
| *Canthidermis maculatus* | unknown | 1 |
| *Balistoides conspicillum* | Daiohzaki, Mie-ken, Japan | 1 |
| *Xanthichthys mento* | unknown | 1 |
| Monacanthidae | | |
| *Stephanolepis cirrhifer* | Daiohzaki, Mie-ken, Japan | 2 |
| *Stephanolepis japonicus* | Daiohzaki, Mie-ken, Japan | 1 |
| *Rudarius ercodes* | Kohchi-ken, Japan | 1 |
| *Navodon modestus* | Hokkaido, Japan | 1 |
| *Alutera monoceros* | Daiohzaki, Mie-ken, Japan | 1 |
| *Pseudalutarius nasicornis* | Borneo | 2 |
| Aracanidae | | |
| *Kentrocapros aculeatus* | unknown | 2 |
| Ostraciidae | | |
| *Ostracion cubicus* | Okinawa-ken, Japan | 2 |
| Tetraodontoidei | | |
| Triodontidae | | |
| *Triodon macropterus* | Okinawa-ken, Japan | 1 |
| Tetraodontidae | | |
| *Fugu rubripes rubripes* | East China Sea | 2 |
| *Fugu pardale* | East China Sea | 2 |
| *Fugu niphobles* | Daiohzaki, Mie-ken, Japan | 1 |
| *Fugu vermiculare porphyreum* | East China Sea | 2 |
| *Fugu vermiculare vermiculare* | unknown | 2 |
| *Lagocephalus lunarius spadiceus* | Daiohzaki, Mie-ken, Japan | 1 |
| *Tetraodon hispidus* | Daiohzaki, Mie-ken, Japan | 1 |
| *Canthigaster rivulata* | Daiohzaki, Mie-ken, Japan | 1 |
| Diodontidae | | |
| *Chilomycterus affinis* | Hachijo Is. Tokyo, Japan | 1 |
| | Malaysia | 1 |
| *Diodon holacanthus* | Hachijo Is. Tokyo, Japan | 1 |
| *Diodon liturosus* | Okinawa-ken, Japan | 1 |
| *Diodon hystrix* | Hachijo Is. Tokyo, Japan | 1 |
| Molidae | | |
| *Mola mola* | Daiohzaki, Mie-ken Japan | 1 |

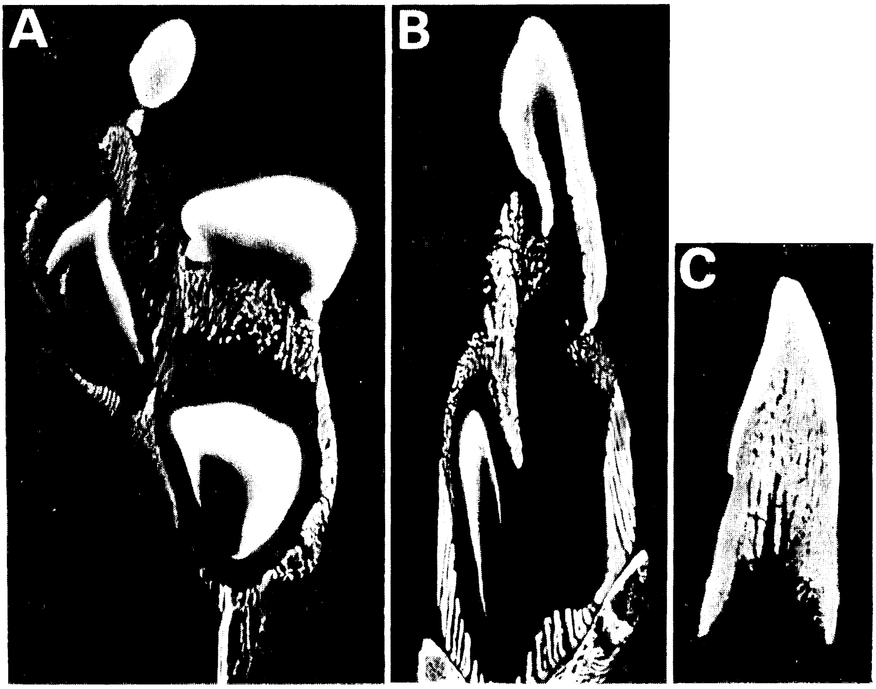

Fig. 1. Microradiograms of the undemineralized ground sections of the teeth of fishes of the Balistoidei. A: *Triacanthus brevirosteris*, Triacanthidae. B: *Balistoides conspicillum*, Balistidae. C: *Ostracion cubicus*, Ostraciidae. ×120.

EMX-SM, equiped with a wave-length dispersive monochrometer. The analyses were performed with an accelerating voltage of 15 kV and specimen current of 0.15 μA (Suga, 1972). The crystals used were a 4-inch RAP crystal for the detection of FK$\alpha$, a 4-inch LiF for CaK$\alpha$, and a 4-inch ADP for MgK$\alpha$.

Quantitative fluoride analysis was carried out by measuring the intensity of FK$\alpha$ emission obtained by the mode of point analysis using the convergent method with fluorapatite (Durango; fluoride concentration estimated by the fluoride solid-membrane electrode method was 2.81%) as a standard sample, as described in a previous report (Suga et al., 1980). A point analysis was made at the surface layer of the enameloid, the middle layer of dentin and the alveolar bone. The average value obtained from three points in the each region is shown in Fig. 4. The fluoride concentration revealed by the electron microprobe is the value of weight per volume bombarded by an electron beam and is not weight per weight of apatite.

The patterns of elemental distributions obtained by line scan analysis were compared in detail with the mineralization pattern revealed by microradiography.

Table 2. Locality and number of specimens from the Acanthuridae of the order Perciformes.

| Species | Locality | Number |
|---|---|---|
| *Acanthurus dussumieri* | Ishigaki Is., Okinawa-ken, Japan | 2 |
| *Acanthurus nigricans* | Ishigaki Is., Okinawa-ken, Japan | 1 |

## Results

**Mineralization pattern of teeth revealed by microradiography** (Figs. 1, 2). The Balistoidei have separate and well-developed chisel-like teeth with long roots (Fig. 1). On the other hand, the Tetraodontoidei except for the Molidae have a knife-shaped plate formed by the fusion of individual teeth (Fig. 2). A longitudinal labio-lingual ground section of the plate showed that the layers of the tooth plate from the early stage of mineralization to the

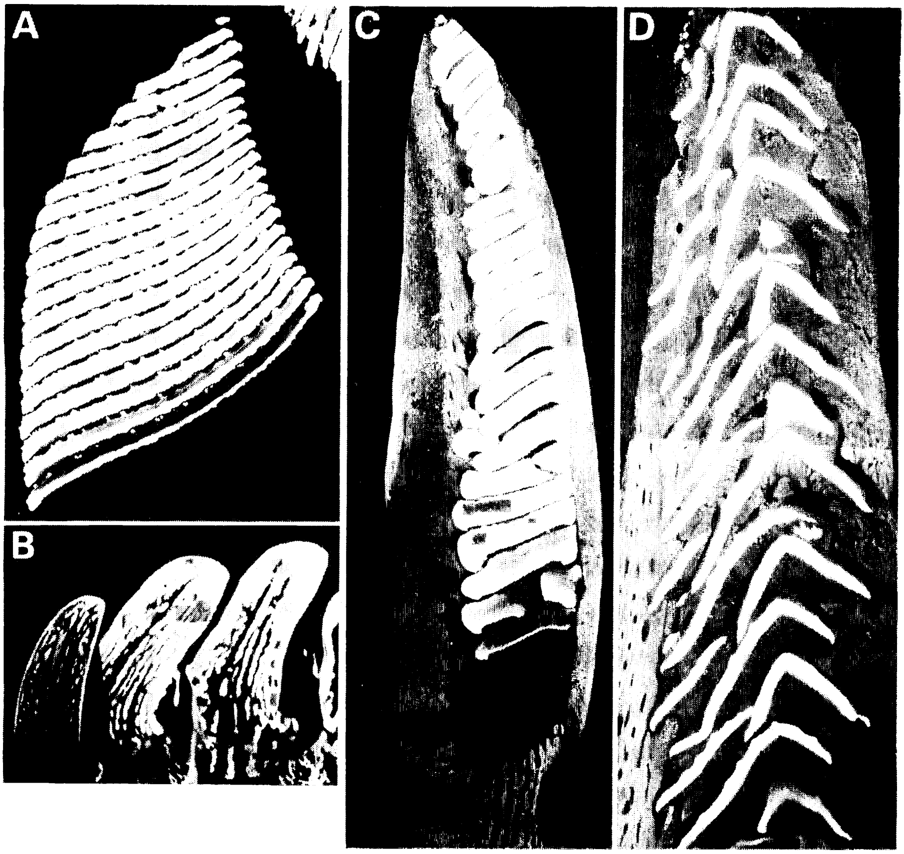

Fig. 2. Microradiograms of the undemineralized ground sections of the teeth of fishes of the Tetraodontoidei. A: *Chilomycterus affinis*, Diodontidae. B: *Mola mola*, Molidae. C: *Fugu vermiculare porphyreum*, Tetraodontidae. D: *Triodon macropterus*, Triodontidae. ×120.

fully formed stage are surrounded firmly by the alveolar bone (Andreucci, 1968). The degree of mineralization of the enameloid was the lowest at the bottom of the layers and increased gradually towards the incisal edge of the plate. It appeared that mineralization was fully accomplished at the middle level of the plate. Dentin was less mineralized and much thinner than matured enameloid.

The form and structure of the teeth of *Mola mola* were quite different from the teeth of the other fish (Andreucci and Britski, 1969). The dentin was composed of sponge-like osteodentin and its coronal surface was covered by a very thin layer of highly mineralized enameloid (Fig. 2B). Therefore, its enameloid could not be used for the elemental analysis. It has been suggested that there is a reductive tendency in the number of teeth which corresponds to the trend in the evolution of this order (Breder and Clark, 1947; Matsuura, 1979).

In the microradiograms, the enameloid of all the fishes examined could be very easily distinguished from the dentin by its very high radiodensity. The progressive and final mineralization patterns of the enameloid were similar in both suborders.

In fully formed enameloid, the degree of mineralization was the highest at the surface layer and decreased gradually towards the enameloid-dentin junction, and, inside the dentin layer, it showed a very sharp decrease. The degree of mineralization of bone was very similar to that of the dentin.

**Elemental analysis by the electron microprobe** (Figs. 3 and 4). The results of a line scan analysis (Fig. 3A) along a line crossing both the enameloid and dentin layers of the fully formed

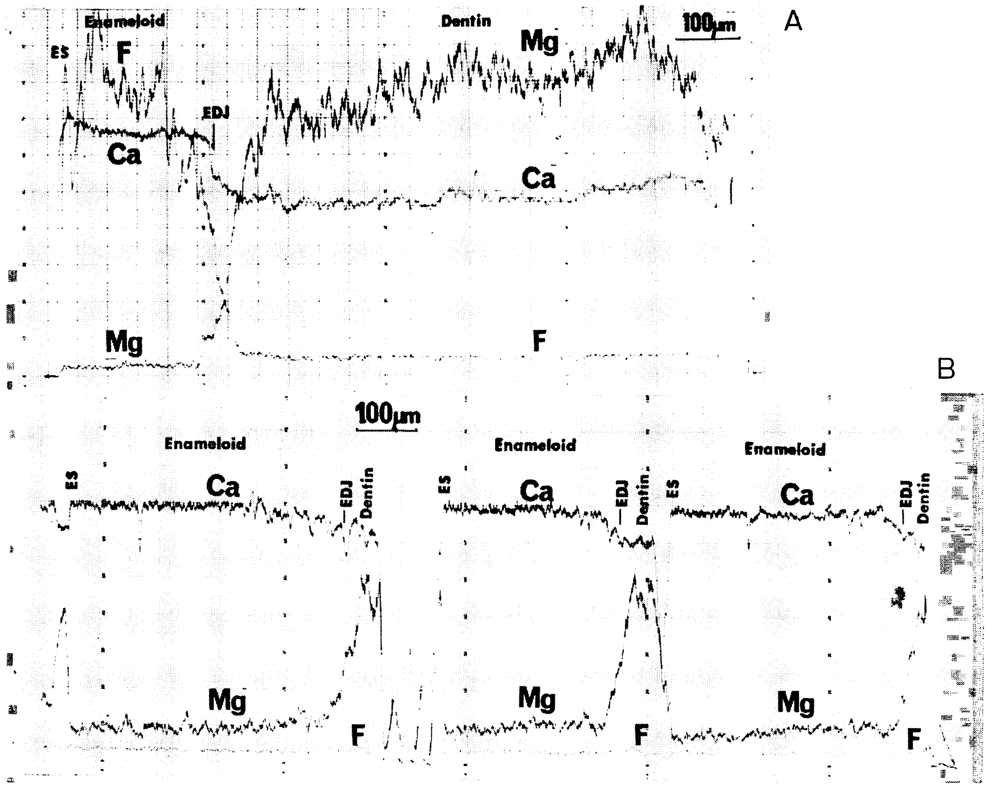

Fig. 3. The distribution of fluoride, magnesium and calcium in the teeth of two fishes, revealed by line scan analysis by the electron microprobe. A: *Alutera monoceros*, Monacanthidae. Line scan analysis was performed at the cusp of crown of the erupted tooth. Fluoride concentration is much higher in the enameloid than in the dentin. B: *Chilomycterus affinis*, Diodontidae. Line scan analysis was performed along the line crossing three layers of fully formed and unerupted tooth. Fluoride concentration is very low in both the enameloid and dentin.

Magnesium concentration is lower in the enameloid than in the dentin, of the teeth of these two fishes. ES, enameloid surface; EDJ, enameloid-dentin junction.

teeth indicated that the fluoride concentration was much higher in the enameloid than in the dentin in all the balistoids examined. Fluoride concentration was the highest at the surface layer of the enameloid and decreased very gradually towards the enameloid-dentin junction and then showed a very sharp decrease in dentin. As described before (Suga et al., 1980), a high concentration of fluoride was observed not only in the erupted enameloid, but also in the developing enameloid at a late stage. Although the fluoride level was very low in the dentin, in the narrow dentin layer adjacent to the enameloid-dentin junction, the fluoride tended to slightly increase in concentration from the pulpal side towards the enameloid-dentin junction (Fig. 3A).

On the other hand, both enameloid and dentin showed very low fluoride concentration in the Tetraodontoidei, except for *Mola mola* (Fig. 3B). The concentration and distribution pattern of calcium and magnesium were almost the same in the Balistoidei and Tetraodontoidei. Calcium concentration was higher in the enameloid than in the dentin and increased slightly from the side of enameloiddentin junction towards the surface. Magnesium showed much higher concentration in the dentin than in the enameloid (Fig. 3A, B).

A quantitative point analysis of fluoride (Fig. 4) indicated that the enameloid of all the Balistoidei specimens contained a very high con-

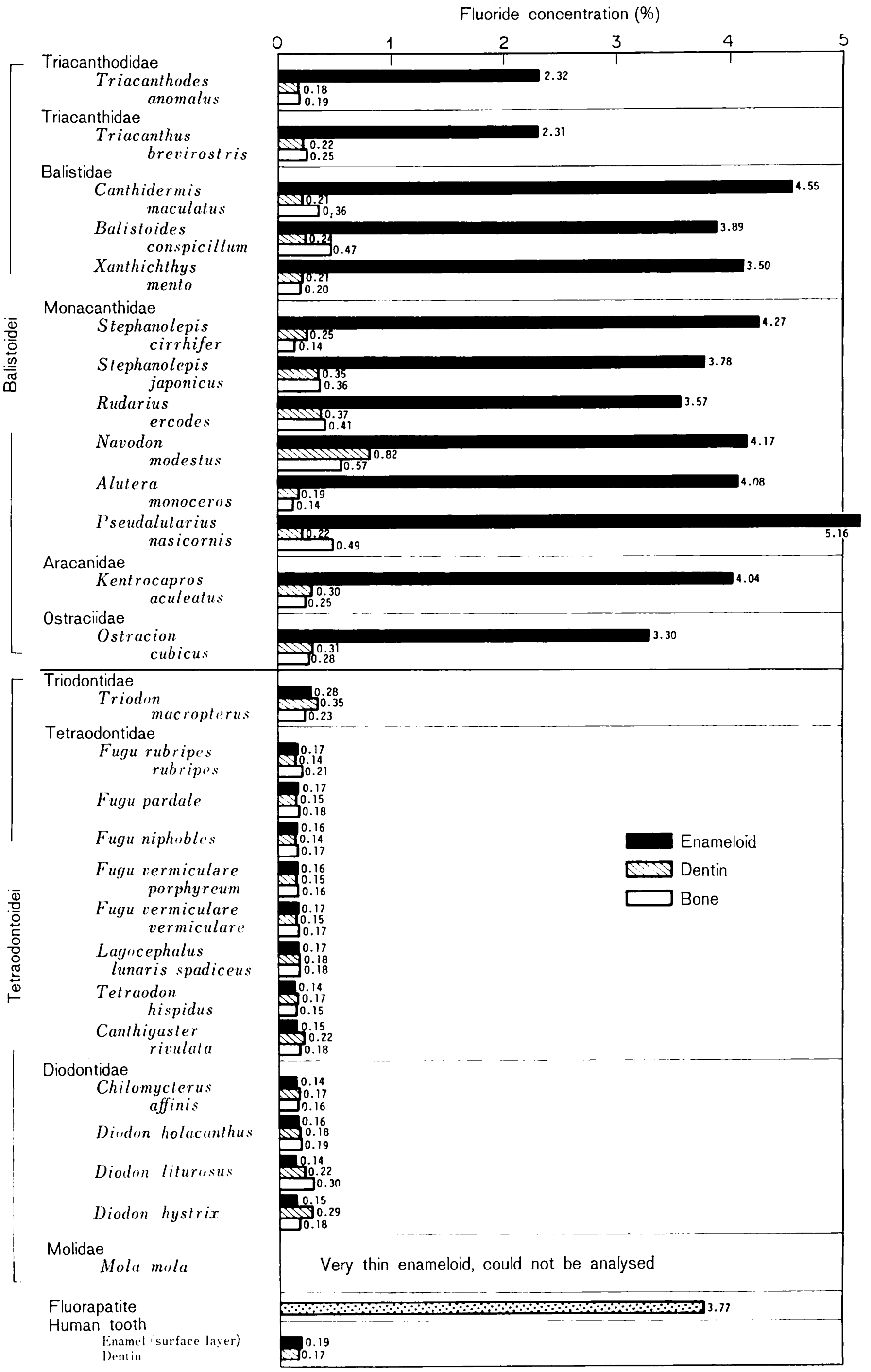

Fig. 4. Histogram showing the fluoride concentration in the enameloid, dentin and bone of the Tetraodontiformes. The fluoride concentration of all the Balistoidei examined was higher than 2.31%, whereas that of the Tetraodontoidei was less than 0.28%, except for the Molidae. The fluoride concentration in matured and unerupted human enamel and its theoretical value in the fluorapatite are also shown.

centration of fluoride ranging from 2.31 to 5.16% whereas the concentration ranged only from 0.14 to 0.28% in the Tetraodontoidei. The enameloid of the Molidae could not be analysed due to its slender width.

Fluoride concentration in the dentin ranged from 0.14 to 0.82% throughout all the fish of this order with a tendency to be slightly higher in the Balistoidei than in the Tetraodontoidei. The concentration in the bone was almost the same in range and tendency as in the dentin.

A specimen of *Chilomycterus affinis* obtained from the Malaysian coast showed almost the same value as a specimen of the same species obtained from the Japanese coast. It contained 0.13% fluoride in the enameloid, 0.16% in the dentin and 0.10% in the bone.

Analysis of two fishes of the Acanthuridae (*Acanthurus dussumieri* and *A. nigricans*) showed that the enameloid contained 3.85 and 3.98%, dentin 0.40 and 0.48% and bone 0.36 and 0.34%, respectively. These values were almost the same as observed in the Balistoidei.

The fluoride concentration in human enamel (the surface layer) and dentin (the middle layer) was 0.19% and 0.17%.

## Discussion

It is generally considered that the fluoride concentration of mammalian teeth is related to that in drinking water (Brudevold and Söremark, 1967). In our earlier study (Suga et al., 1976), we also assumed that the concentration of fish enameloid is related to that of the environmental water. This was based on data on two marine fishes belonging to the Perciformes which showed higher fluoride concentrations than freshwater fishes belonging to the Cypriniformes. Fluoride concentration in the ocean is about 1.3 p.p.m. throughout the world (Greenhalgh and Riley, 1963), whereas that of the fresh water in lakes and rivers is usually about 0.26 p.p.m. (Matuura and Kokubu, 1972).

However, the present investigation shows that there is a marked difference in the fluoride concentration in the enameloid between the Balistoidei and Tetraodontoidei despite the fact that both are marine fishes. The concentration in the Balistoidei is almost the same as or higher than fluorapatite (F: 3.77%), whereas that of the Tetraodontoidei is as low as that of human enamel (about 0.19% at the surface layer).

The results of the present investigations were plotted into the phylogenetic tree of the Tetraodontiformes based upon osteological features (Tyler, 1980) (Fig. 5). It should be also noticed that the enameloid of the family from which this order may have derived (the Acanthuridae of the Perciformes) contained high fluoride. These results strongly support our recent speculation that there is a phylogenetic relationship between fluoride concentration and enameloid (Suga et al., 1977, 1979, 1980). It follows from this that there is some special fluoride concentrating mechanism in the balistoid fish enameloid, although we do not know

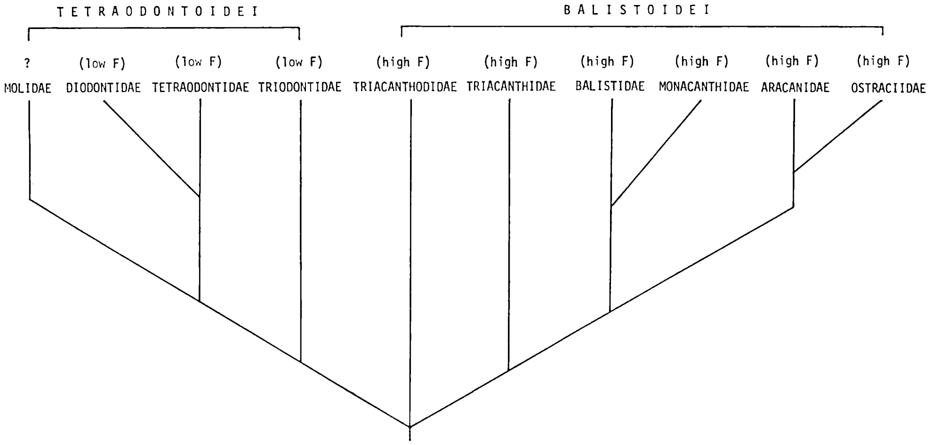

Fig. 5. The results of the present investigation were plotted with the phylogenetic tree of the order Tetraodontiformes proposed by Tyler (1980) based upon osteological characters.

exactly what the mechanism is. If this is accepted, it also follows that the fluoride concentrating mechanism has been lost during the evolution of the Tetraodontoidei.

Although the concentration is very low in the dentin of the Balistoidei, it tends to increase gradually from the pulpal side to the enameloid-dentin junction, in the narrow layer adjacent to the enameloid-dentin junction. This observation seems to support our previous view that the ectodermal enameloid-forming cells have a special ability to concentrate fluoride in the enameloid (Suga et al., 1978, 1980).

Crystallographic analyses by X-ray diffraction and infrared spectroscopy indicated that fluoride in the enameloid is principally incorporated in the apatite. This results in the greater crystalite size, reduction in carbonate content, and systematic decrease in the a-axis lattice parameter (LeGeros and Suga, 1980).

Most tetraodontiform fishes usually feed on invertebrates such as crustaceans, mollusks, echinoderms, etc., and small fish. There seems to be no appreciable difference in the feeding habits of the two suborders (Hiatt and Strasburg, 1960), except for the fish of the Molidae (Fraser-Brunner, 1951). This fact seems to indicate that the fluoride concentration in the enameloid is independent of diet and feeding habits.

The fact that the teeth of one species, *Chilomycterus affinis*, obtained from the two different regions showed almost the same value of fluoride concentration also support our speculation that fluoride concentration in the enameloid is independent of the environment.

Since fluoride concentration in the dentin and bone of the Balistoidei is slightly higher than that of the Tetraodontoidei, one cannot exclude the possibility that there may be another systemic fluoride concentrating mechanism in the Balistoidei, although at present we have no sufficient information to explain such a mechanism.

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## フグ目魚類の歯質中のフッ素含量と系統との関係

須賀昭一・和田浩爾・小川正明

フグ目の魚の歯質，ならびに骨質中に含まれるフッ素 (F) の定量分析をエレクトロンマイクロプルーブによって行った．フグ目は比較的原始的なモンガラカワハギ亜目とより進化したフグ亜目とからなる．前者に属する魚 (6 科 13 種) のエナメロイド中のフッ素含量は 2.31% 以上であるのに対して，後者に属する魚 (4 科 13 種) のそれは 0.22% 以下であった．又，フグ目の祖先型と考えられているスズキ目のニザダイ科の魚 (2 種) のエナメロイドも 3.85% 以上のフッ素を含んでいた．

検索したすべての魚の象牙質と骨中のフッ素含量はそれぞれ 0.82%，0.57% 以下であった．

以上の所見からエナメロイド中のフッ素含量は環境水中のフッ素含量よりも，魚の系統発生と密接に関係していると考えられる．又，モンガラカワハギ亜目の魚ではそのエナメロイド形成細胞にフッ素濃縮能が存在することが想像されるが，フグ亜目の魚ではその能力が進化の過程で失われたのではないかと考えられる．

(須賀・小川：102　東京都千代田区富士見 1–9–20 日本歯科大学病理学教室；和田：517–05　三重県志摩郡阿児町賢島　養殖研究所賢島支所)