In vivo and in vitro study of ^{59}Fe uptake in developing rat molars

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Autoradiographic methods were used to study ^{59}Fe uptake in vivo in mineralizing tissues of young rats. Localization of ^{59}Fe was observed in the ameloblastic layer of molars. In vitro studies were performed which demonstrated that ^{59}Fe uptake in developing rat molar enamel was limited by the metabolic activity of the cells of the enamel organ.

Key-words: Mineralization; physiology

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Most of the investigative work concerning the uptake and distribution of iron in teeth has been focused on the condition in the rodent incisor where a specific iron pigment is deposited in the enamel. The nature and timing of the deposition of the pigment, as well as the influence of a variety of factors on the process, have been well defined. (For review, see (7).) It has also been shown that trace amounts of iron are found in the enamel of erupted teeth in several species, including humans, although no iron pigment is normally found in these teeth (2, 3, 5, 8, 9, 11, 12, 13).

The topical application of ferric chloride and ferrous sulfate, or the addition of these salts to the water and diet of hamsters and rats with mature, erupted teeth, has been shown to reduce dental caries in experimental situations (4, 10). Glass et al. (6) have shown that the water in a community with low caries prevalence, as compared with a similar community with twice the caries rate, had significantly lower iron content. However there were differences in the concentration of other elements as well and the role of iron in reducing the prevalence of caries is not clear. On the other hand, Besic et al. (2) have reported that increased concentrations of iron in enamel are positively related to decreased enamel solubility in acid.

It appears that, apart from conditions relating to the rat incisor, little is known about the uptake and effects of iron in the developing tooth, especially in the forming enamel. The present investigation was undertaken in an effort to study patterns of iron uptake in the developing bones and teeth of rats in vivo using autoradiographic methods. In vitro methods were employed to study factors which influence the uptake of iron in the enamel of developing rat molars.

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MATERIALS AND METHODS

In vivo experiments

Three series of four littermate Sprague-Dawley rats were used. At eight days of age, each animal was injected intraperitoneally with a dose of 2 μCi/g body weight of $^{59}Fe\ Cl_3$ solution (spec. act. 15 mCi/mg Fe, New England Nuclear, Boston, Mass., USA). The animals were killed at 1, 4, 24 and 96 hours after injection of the tracer, placed in a solution of carboxymethyl cellulose (CMC) and frozen by immersion in hexane cooled with dry ice (−75 °C). Whole body frozen sections 5 μm thick were taken according to the method of Ullberg (14). After the sections were freeze-dried for 2 days, they were pressed against X-ray film (Industrex AA, Eastman Kodak Co., Rochester, NY, USA) and exposed for 2–3 weeks. ^{59}Fe uptake patterns were evaluated by superimposing the autoradiographs over the unstained sections and viewing them through a stereo microscope.

In vitro experiments

The maxillary first and second molars were removed as a unit, with the surrounding enamel organ intact, from 8-day-old Sprague-Dawley rats, transferred to a grid culture system and cultured in a defined medium (Ml99) supplemented with 50 μg/ml of ascorbic acid and 5 μg/ml of chloramphenicol (1). The cultivation was carried out in a 95 % O_2 + 5 % CO_2 gas mixture at 37 °C and 100 % humidity. After an initial incubation period of 1 hour, the explants were transferred to prewarmed medium containing 1 μCi/ml of $^{59}FeCl_3$ solution and cultured for periods of 1, 4 and 8 hours.

To evaluate the role of the cells of the enamel organ in regulating iron movement into the enamel, the following experimental conditions were imposed: (1) a metabolic inhibitor, 5 mM 2,4-dinitrophenol (DNP) was added to the culture medium; (2) the explants were maintained in medium for 10 minutes at 70 °C before transfer to the culture system; or (3) the enamel organ was removed from the teeth prior to cultivation. The metabolic inhibitor was added to the medium during the initial cultivation as well as to the ^{59}Fe containing medium, thus permitting it to act on the cells before exposure to the tracer.

Upon termination of the culture period, the specimens were rinsed in ice-cold saline and frozen quickly in a solution of CMC. Freeze-sectioning and autoradiographic procedures were the same as used in the in vivo experiments.

Each time interval included 3 control and 4 each of DNP-treated, heat killed and stripped specimens.

RESULTS

In vivo series

The whole body autoradiography sections from the animal terminated at one hour showed that most of the injected dose of ^{59}Fe was still in the peritoneal cavity in the area of the injection site. Slight uptake could be seen in the liver, bone marrow and blood.

After 4 hours the uptake in the liver and bone marrow had greatly intensified, and concentrations in the blood had increased moderately (Fig. 1). A very slight amount of activity was seen in the long bones in a narrow line along the epiphyseal plate. The postsecretory ameloblasts and the most mature areas of enamel in the incisor teeth exhibited an intense localization of ^{59}Fe. In the molars a slight, but distinct, line of activity was observed in the ameloblastic layer, most prominently associated with those cells in the postsecretory phase (Figs. 1, 2). No significant localization of the tracer could be seen in the enamel, dentin or pulp.

At 1 and 4 days, the same general pattern which was observed after four hours persisted

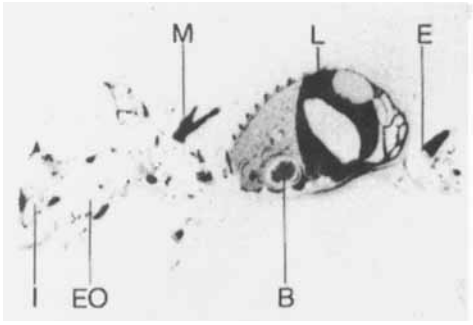


Fig. 1. Whole body autoradiograph of an 8-day-old rat injected intraperitoneally with ^{59}Fe and killed 4 hours later. Heavy uptake of tracer is seen in the liver (L), bone marrow (M), and developing incisor teeth (I). Moderate uptake is seen in blood (B). Low concentrations of ^{59}Fe can be seen in the epiphyseal plate of long bones (E) and the enamel organ of the molar teeth (EO). Mag. 1 x.

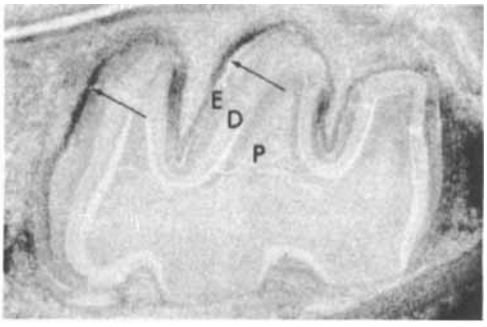
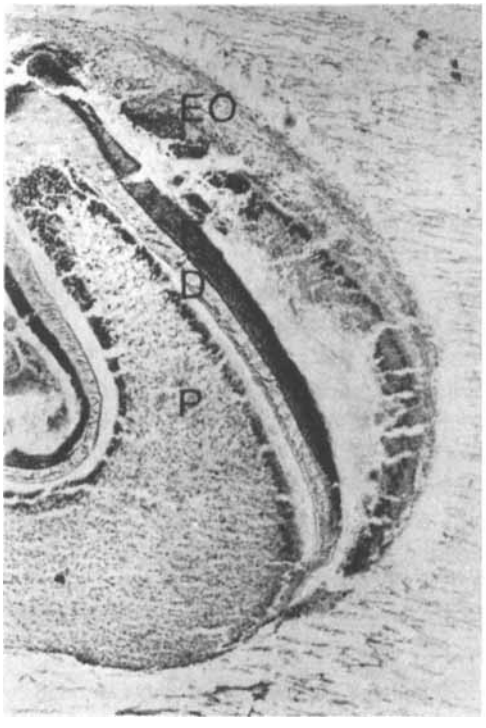


Fig. 2. An unstained, freeze-dried section of an upper first molar from an 8-day-old rat injected intraperitoneally with ^{59}Fe and sacrificed 4 hours later. The contact autoradiograph produced from the section is superimposed. The arrows indicate tracer localization in the postsecretory ameloblasts. Enamel (E), dentine (D) and pulp (P). Mag. 25 x.



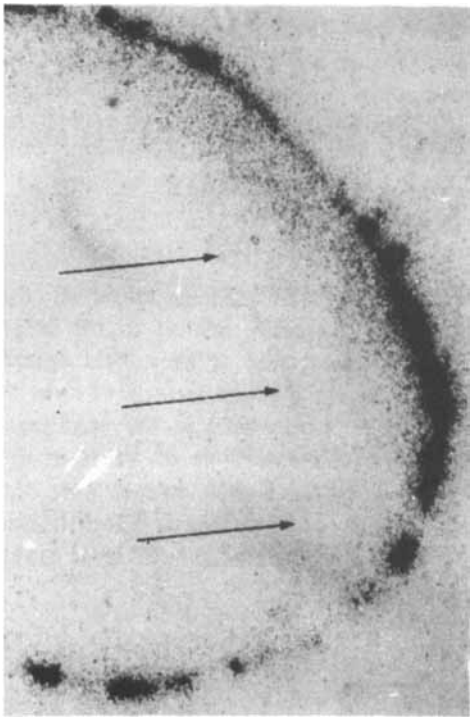


Fig. 3 (left) An H & E stained, freeze-dried section of a maxillary first molar, taken from an 8-day-old rat and cultured for 4 hours in medium containing ^{59}Fe. Enamel organ (EO), enamel (E), dentine (D) and pulp (P). Material surrounding the enamel organ is the carboxymethylcellulose imbedding medium, not tissue. Mag. 50 x.
(right) An autoradiograph produced from the same section. Uptake of ^{59}Fe is confined to the enamel organ. Arrows indicate the enamel surface.

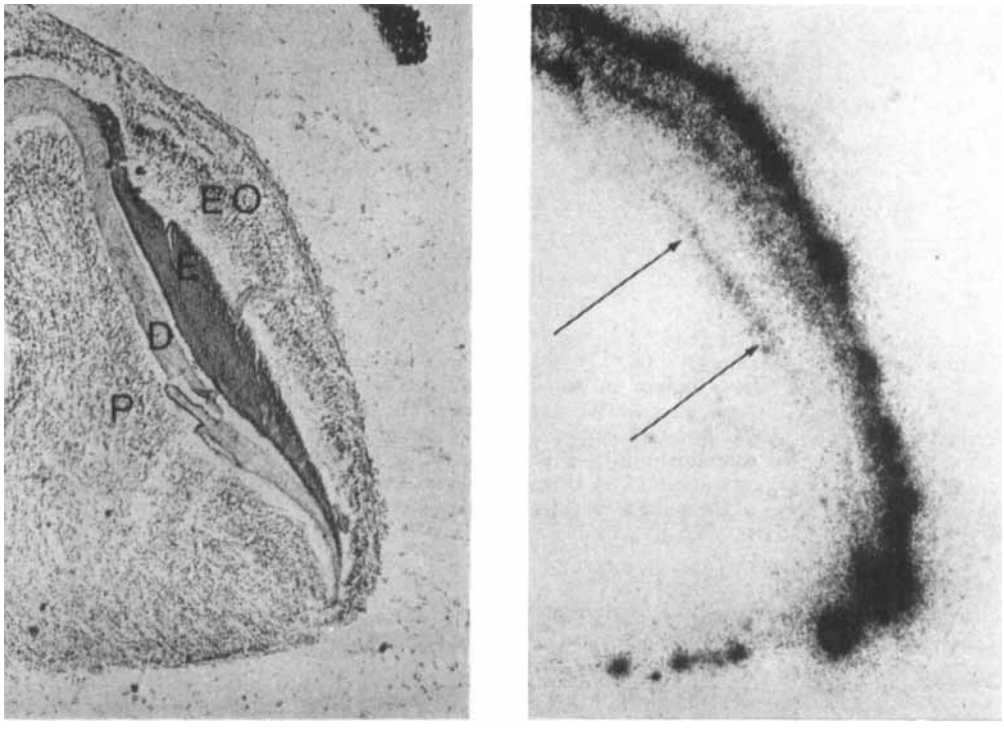


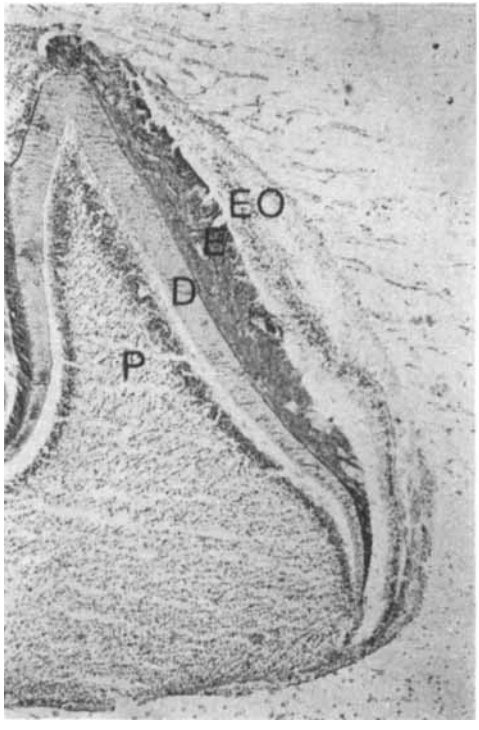
Fig. 4 (left) An H & E stained, freeze-dried section of a maxillary first molar, taken from an 8-day-old rat and cultured for 4 hours in medium containing 5 mM DNP and ^{59}Fe. Enamel organ (EO), enamel (E), dentine (D), and pulp (P). Mag. 50 x.
(right) An autoradiograph produced from the same section. ^{59}Fe has penetrated the enamel organ and has entered the enamel surface (arrows).

except that ^{59}Fe concentrations in the blood had increased relative to the other tissues. There seemed to be a slight uptake of ^{59}Fe on the growing surfaces of bone in addition to localization in the epiphyseal plate. The concentrations of tracer in the incisor ameloblasts and enamel were the most intense of all tissues, and activity was still obvious in the ameloblastic layer of the molars.

In vitro series

After 1 hour in culture, the autoradiographs showed that uptake of ^{59}Fe was confined to the surface of the outer enamel epithelium of all the molar explants, whether cultured under control or experimental conditions. In the explants cultured under control conditions for 4 hours, uptake of ^{59}Fe had occurred in the ameloblastic layer, but only a barely detectable amount of tracer was found at the enamel surface (Fig. 3). In the explants which had been metabolically inhibited with DNP, or had been heat killed, the ^{59}Fe had penetrated the enamel organ and entered the surface enamel (Figs. 4, 5). In the teeth which had been stripped of the enamel organ, uptake of tracer in the surface enamel was observed (Fig. 6).

When the culture period was extended to 8 hours, uptake patterns under the various experimental conditions remained the same as those seen at 4 hours. Concentrations of ^{59}Fe in the respective areas of localization had increased moderately.



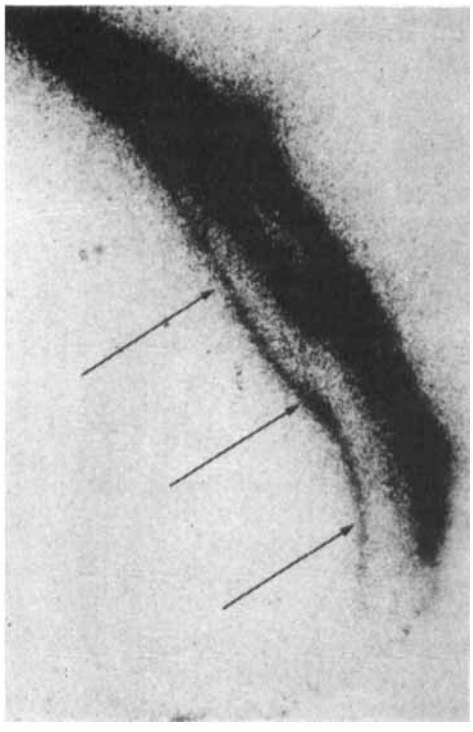


Fig. 5 (left) An H & E stained, freeze-dried section of a maxillary first molar, taken from an 8-day-old rat, heat killed, and cultured for 4 hours in medium containing ^{59}Fe. Enamel organ (EO), enamel (E), dentine (D) and pulp (P). Mag. 50 x. (right) An autoradiograph produced from the same section. ^{59}Fe has penetrated the enamel organ and entered the enamel surface (arrows).

DISCUSSION

The general distribution of ^{59}Fe observed in the in vivo component of this study was, with one exception, much as anticipated. Relatively high activity in the liver, bone marrow and blood was consistant with the known metabolism of iron in the body. Uptake in bone was minimal and seemed to be confined to areas of growth at the epiphysis and on the surfaces. Localization occurred at a rather precise time of growth in this tissue, and may have been associated with the formative cells.

The high concentrations and pattern of distribution of the tracer in the incisor ameloblasts and enamel correlated well with the findings of other investigators concerning deposition of iron pigment in rat incisor enamel (7). However, Halse (7) did not find deposition of pigment in the enamel of rat incisors until after 10 days of age, but the autoradiographs obtained in this study indicated uptake of ^{59}Fe in the ameloblasts and enamel at 8 and 9 days.

The unexpected finding related to low, but definite, concentrations of iron in the ameloblastic layer of the molars. No iron pigment, or other specific role for the element, has been identified with these teeth. Iron is found in trace amounts in mature enamel including rat molar enamel (2, 3, 5, 8, 9, 11, 12, 13); Torell, 1955; but the ^{59}Fe observed in this study appeared to be confined to the enamel organ and did not enter the molar enamel. Iron could have been associated with certain enzymes in

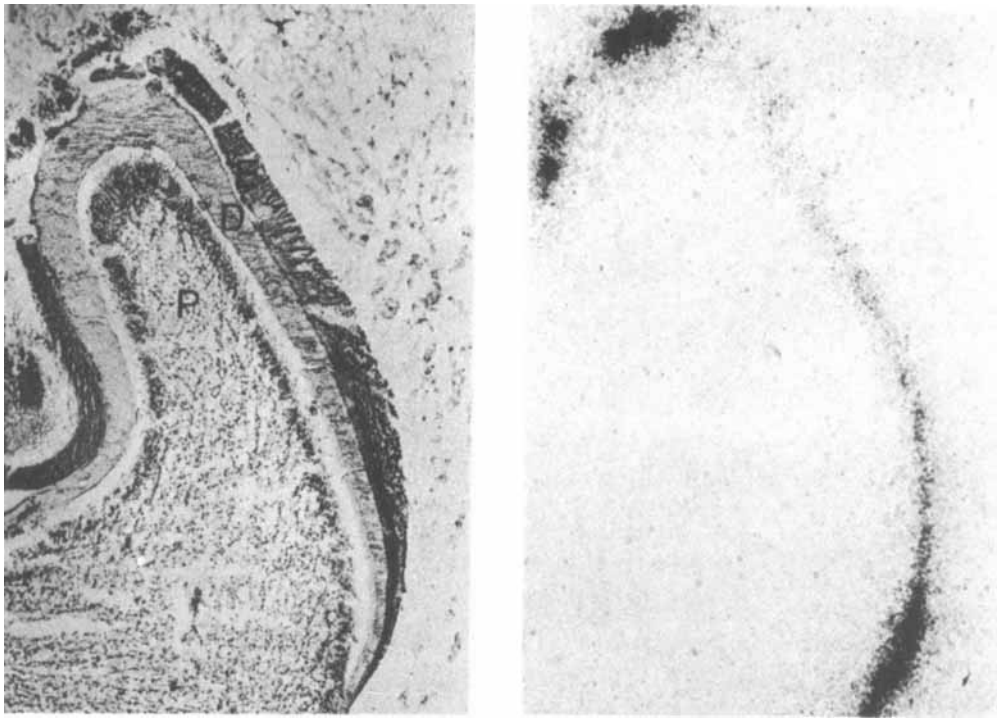


Fig. 6 (left) An H & E stained, freeze-dried section of a maxillary first molar, taken from an 8-day-old rat, stripped of the enamel organ, and cultured for 4 hours in medium containing ^{59}Fe. Enamel (E), dentine (D), and pulp (P). Mag. 50 x.
(right) An autoradiograph produced from the same section. ^{59}Fe is localized in the surface enamel.

the molar ameloblasts, such as the cytochrome enzymes and succinic dehydrogenase.

Results of the in vitro series indicated that, in the control explants, ^{59}Fe uptake was confined to the enamel organ except for a barely detectable concentration at the surface of the developing enamel. The pattern was similar to that observed in vivo. Since chemical metabolic inhibition or heat killing resulted in penetration of ^{59}Fe through the enamel organ and into the surface enamel, it appears that metabolic activity of the cells of the rat molar enamel organ is required to restrict uptake of iron in the developing enamel.

Uptake in the surface of the enamel in the explants which had been stripped of enamel organ indicated that the tracer was readily absorbed by the enamel when allowed to come in direct contact. Torell (13) has described the uptake of iron from saliva by the enamel of erupted teeth, indicating that mature enamel has an affinity for iron. Considering the virtual absence of ^{59}Fe in the developing enamel of the in vivo sections, and in vitro control specimens in the current study, it would appear that most of the iron found in the enamel of erupted teeth is acquired post eruptively from the saliva. Metabolic activity of the cells of the enamel organ appears to limit uptake of iron during enamel development.

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