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RADIOPATHOLOGY OF AMERICIUM 241

II. Uptake in the developing teeth of rats

by

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A previous study of the distribution of ^{241}Am in adult mice indicated that the dental pulp accumulated considerable amounts of this radionuclide (HAMMARSTRÖM & NILSSON 1970). Investigation on the distribution in the teeth of other actinide radioelements such as ^{239}Pu and ^{228}Th (JEE & ARNOLD 1960, ULLBERG et coll. 1962) have revealed that these elements are deposited in the newly formed dentinal surface of the pulp chambers although no uptake in the soft tissues of the dental pulp has been reported. It was therefore considered of value to study more in detail the uptake of ^{241}Am in the teeth. The distribution of ^{241}Am in the developing enamel was also investigated. So far there seems to be no detailed report on the distribution of any actinide element in the developing enamel.

Material and Methods

Four 10-day-old rats of the Sprague-Dawley strain were injected intraperitoneally with 0.26 ml of ^{241}Am citrate solution to give an individual dose of

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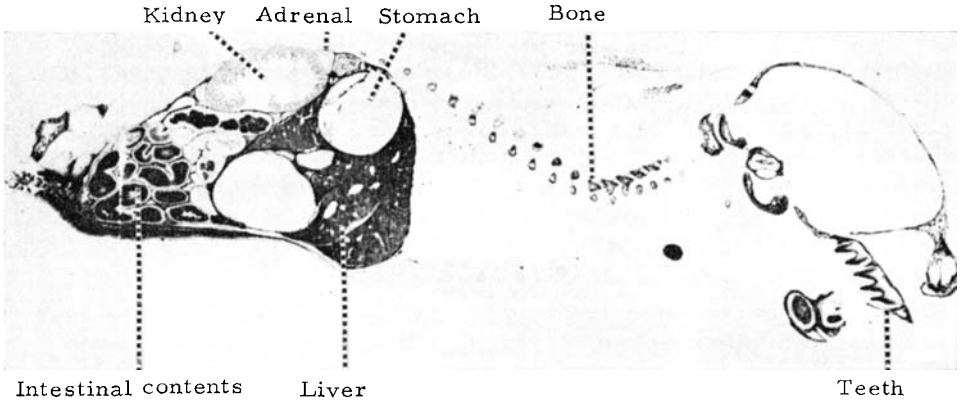


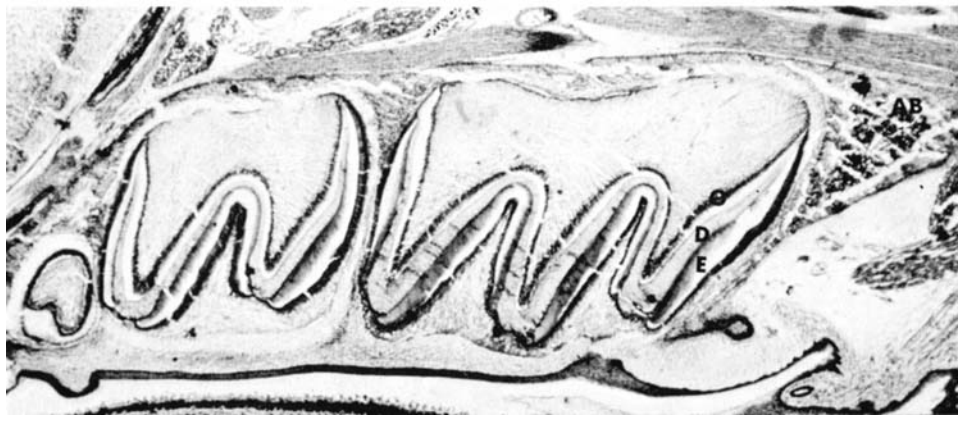
Fig. 1. Whole body autoradiography. Distribution of ^{241}Am in an 11-day-old rat 24 hours after intraperitoneal injection. Black areas indicate high concentration of radioactivity. Deposition in bone and teeth; high concentration in the liver and the intestinal contents; low concentration in the kidney, and hardly any radioactivity in the adrenal cortex.

about 1 μCi. One rat was killed at respectively 1 hour, 4, 24, and 96 hours after injection by immersion in a mixture of solid carbon dioxide and hexane (— 75° C).

Whole body autoradiography. Sagittal (20 μ thick) sections through the whole frozen animals were cut and dried in a freezebox (— 10° C). The autoradiographic exposures were made by apposition against a roentgen film (Structurix, Gevaert). The exposure time was about 4 weeks for sections of animals of the different survival periods. After exposure, the sections were separated from the roentgen films and stained with hematoxylin and eosin (ULLBERG 1954, 1958). The films were developed, fixed and rinsed.

Microradiography. Twelve (10 μ thick) sections of the head of the rats with survival periods of 1 day and 4 days were cut in a freezebox (— 10° C) and placed on Scotch tape with a polyvinyl chloride backing (No. 688, Minnesota Mining and Manufacturing Co). The sections were dried in the freezebox (— 10° C) and dry-mounted on glycerine-treated nuclear emulsion plates (emulsion thickness 10 μ, Ilford G-5). After exposure for 4 weeks, the Scotch tape was dissolved in xylene, the nuclear emulsion plates developed, fixed and rinsed, and finally the sections still remaining on the emulsion surface were stained with hematoxylin and eosin. The method has been described in detail by HAMMARSTRÖM et coll. (1965).

a



b

Fig. 2. a) Detail of the whole body autoradiogram of the same rat as in fig. 1. b) Corresponding section stained with hematoxylin and eosin. Marked uptake in odontoblasts (O) and pulp surfaces of the dentine (D); low concentration at the surface of the enamel (E); the concentration in the odontoblasts and dentine seems to equal that of the alveolar bone (AB).

Results

The absorption of the intraperitoneally injected ^{241}Am was slow. Radioactivity at 1 hour after injection could be registered only in the peritoneal cavity and the liver. After 4 hours, the concentration in the blood was high, and thus the injected americium was distributed throughout the whole organism although there was no uptake in the skeletal tissues. After 1 and 4 days, however, it lay in the

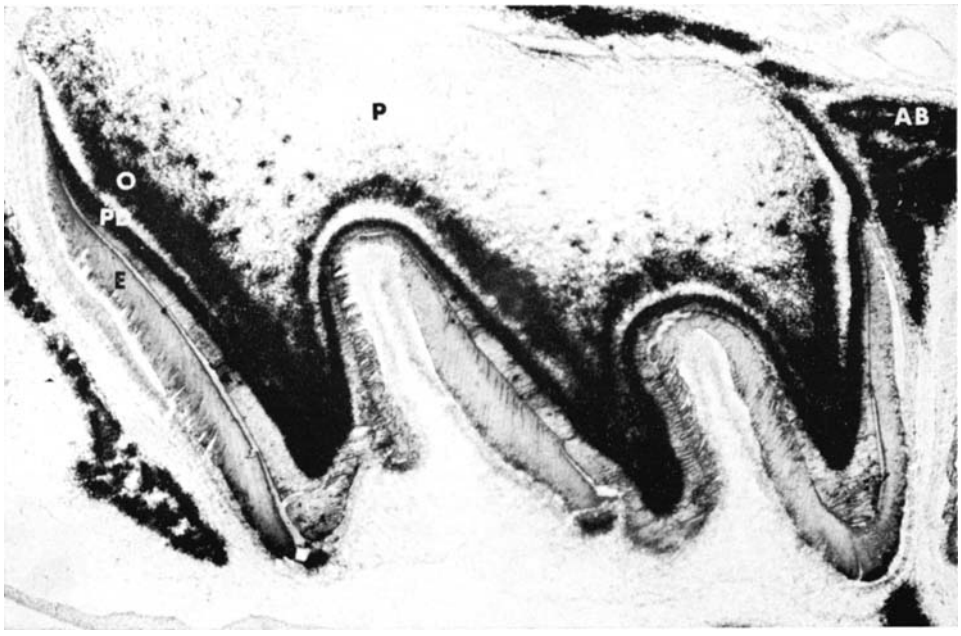


Fig. 3. Microautoradiogram. Distribution of ^{241}Am in the first upper molar of an 11-day-old rat 24 hours after intraperitoneal injection. Uptake in the odontoblasts (O), predentine (PD) and alveolar bone (AB); some spots with high concentration are spread in the pulp (P); there is hardly any uptake at the surface of the enamel (E).

dental tissues and in the periosteal and endosteal surfaces of the bone. The distribution in the body was similar to that in adult mice after intravenous injection, which has recently been described (HAMMARSTRÖM & NILSSON 1970). It was noted that ^{241}Am did not accumulate in the adrenal cortex of young rats, as it did in adult mice (Fig. 1).

In the developing molar teeth, the americium was accumulated in the dental pulp mainly in the odontoblasts, and these cells displayed about the same concentration as that of the surface of bone (Fig. 1). The americium in the odontoblasts was partly located in the distal part of the cells close to the dentine and partly on the pulpal side of the nuclei. However, this latter uptake might also have been located in the pulpal tissue close to the odontoblasts and not within those cells. There was in addition marked accumulation in the predentine 24 hours after injection (Fig. 2). Three days later this zone of radioactivity appeared in the mineralized dentine. The americium seemed to remain unchanged in the odontoblasts during the four days of investigation.

Little of the ^{241}Am was taken up in the developing enamel. Twenty-four

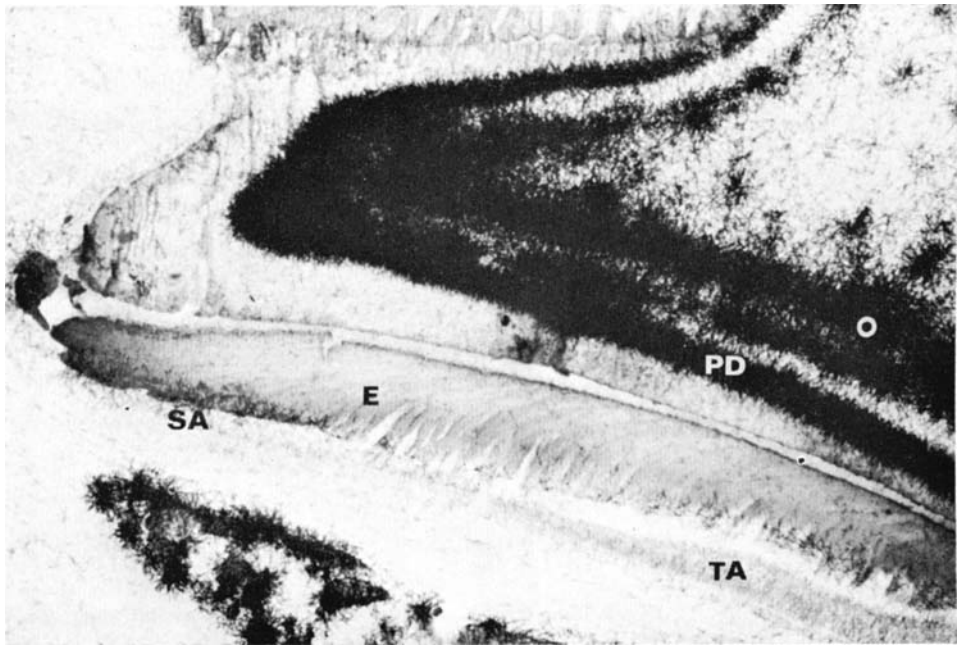


Fig. 4. Detail of section shown in fig. 3, indicating superficial uptake of ^{241}Am in the enamel (E). The concentration is low under the tall ameloblasts (TA) but increases under the short ameloblasts (SA) towards the tip of the cusp; the accumulation in the odontoblasts (O) seems to be divided into proximal, distal and predentinal (PD) parts.

hours after its administration, only faint autoradiographic traces at the surface of the enamel and in the ameloblasts were evident (Fig. 3). The concentration at these sites was markedly lower where matrix formation occurred than in the areas where the enamel had attained its final thickness (Figs 2 and 4).

Four days after injection of americium some radioactivity was seen in a wide zone in the enamel about half-way to the dentine enamel junction (Fig. 6). It should, however, be kept in mind that at all the intervals studied the concentration in the enamel was low in comparison with that in the developing dentine and odontoblasts.

Discussion

The present investigation has confirmed the previous observations (Hammarström & Nilsson 1970) of a marked accumulation of ^{241}Am in the cells of the dental pulp. None of the other actinide radioelements studied have been reported

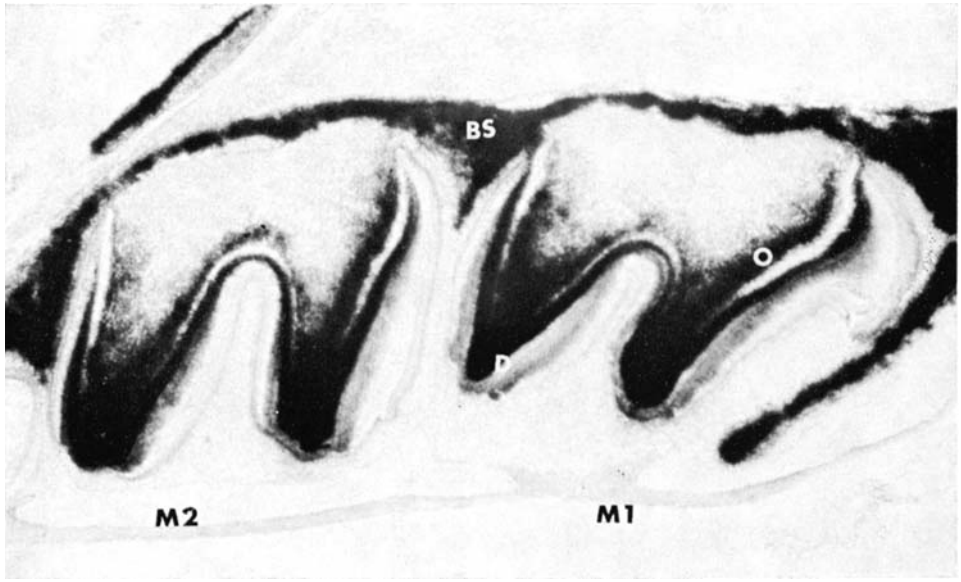


Fig. 5. Microautoradiogram. Distribution of ^{241}Am in the first (M1) and second molar teeth (M2) of a 14-day-old rat 4 days after intraperitoneal injection. High concentration of radioactivity in the pulpal surface of the dentine (D), in the odontoblasts (O) and the interdental bone septum (BS).

to have accumulated in the odontoblasts (Jee & Arnold 1960). It is still unsettled whether this accumulation is specific for americium or whether the methods used for the localization of other actinides have failed to detect it in these cells.

The autoradiographic techniques used in the present investigation were such that tissue was never in contact with any liquid medium before autoradiographic exposure, so that redistribution or isotope loss before recording in the photographic emulsion was prevented. The marked accumulation of americium in the odontoblasts may indicate that americium is taken up in the osteoblasts as well, since these cells are metabolically and functionally similar. No such uptake appears, however, to have been reported.

It might be expected that the high-energy alfa-radiation of americium would induce neoplastic growth of the odontoblasts and that special attention should be given to the late biologic effect of ^{241}Am on the dental pulp. It should, however, be remembered that the mature odontoblasts are highly radioresistent (Kalnins 1954). Injection of ^{239}Pu in dogs has previously been shown to stimulate secondary dentine formation but no tumours developed (Jee & Arnold 1960).

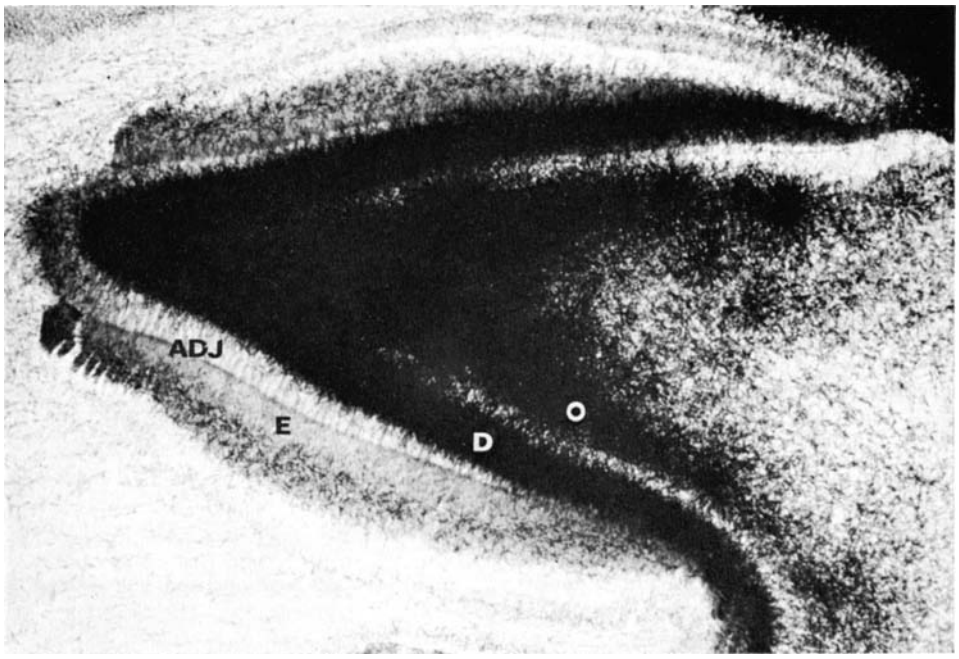


Fig. 6. Microautoradiogram 4 days after injection. The americium is located in the enamel (E) about half-way to the amelodentine junction (AD). The concentrations in the odontoblasts (O) and in the dentine (D) remain high.

Americium is selectively concentrated at the resting and resorbing surfaces of bone (Taylor et coll. 1961, Herring et coll. 1962) and good conformity has been noted between areas with a marked concentration of americium and those with a strong PAS-positive reaction (Herring et coll. 1962). It is therefore interesting to note that the concentration of americium in the enamel increased at the stage of maturation during which organic material and water is removed and minerals are deposited (Deakins 1942, Weinmann et coll. 1942, and others). The surface area, where americium appeared 24 hours after injection, has proved to be PAS-positive (Suga & Gustafson 1963).

The mechanism of binding of americium to the skeletal tissues is still obscure. The fact that there was a low concentration of ^{241}Am in the developing enamel is a good illustration implying that the mineral component is of little or no importance in the binding mechanism. Recent investigations have indicated that it is bound in bone to some glycoproteins (Herring et coll. 1962, Chipperfield & Taylor 1968). Whether this holds also for its binding to the dental tissues remains to be settled. The pattern of deposition of ^{241}Am in the enamel indicated

no definite relation to protein matrix deposition or mineral deposition as indicated by autoradiography of labelled amino acids and radiocalcium (BELANGER 1957, GREULICH & SLAVKIN 1965, HAMMARSTRÖM & LINGE, to be published).

The latency in the accumulation of ^{241}Am at the surfaces of bone and in odontoblasts and ameloblasts may suggest that it be incorporated into a larger molecule with an affinity for these tissues. Few substances have a marked affinity for the postsecretory ameloblasts, the metabolism of which is very little known. The only metal so far known to accumulate in the ameloblasts of rat molar teeth during maturation is iron (HAMMARSTRÖM & JONSSON, in preparation). It is therefore interesting that plutonium, and probably americium as well, are transported in the blood bound to transferrin (BOOCOCK & POPPLEWELL 1965, STOVER et coll. 1968). This may indicate that the accumulation in the ameloblasts occurs in the form of an americium-transferrin complex.

SUMMARY

Young rats were injected intravenously with ^{241}Am citrate solutions and investigated at different times by whole body autoradiography and microradiography. The techniques are described and the findings discussed in detail.

ZUSAMMENFASSUNG

Junge Ratten wurden mit ^{241}Am Citrat injiziert und in verschiedenen Zeitabständen mittels Körperautoradiographie und mittels Mikroradiographie untersucht. Die Methodik und die Resultate werden detailliert beschrieben.

RÉSUMÉ

Les auteurs ont injecté à de jeunes rats par voie intraveineuse des solutions de citrate ^{241}Am et les ont examinés à différentes dates par auto-radiographie et microradiographie du corps entier. Ils décrivent les techniques et étudient en détail les résultats.

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