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AUTORADIOGRAPHIC LOCALIZATION OF VANADIUM PENTOXIDE ($V_2^{48}O_5$) IN DEVELOPING TEETH AND BONES OF RATS

by

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During the last ten years several investigations have been published concerning the caries-inhibiting effect of vanadium pentoxide. However, the results from these investigations differ and in several respects are contradictory. *Manly & Bibby* (1949) found that V_2O_5 was active in reducing acid solubility of enamel. *Rygh* (1949), *Geyer* (1953), and *Tank & Storvick* (1960) found that vanadium reduced the dental caries rates significantly. *Hein & Wisotzky* (1955) and *Muhler* (1957), however, came to the opposite conclusion in their experimental studies (cf. the review by *Underwood,* 1956).

In a study of the general gross-distribution pattern of vanadium pentoxide in the body of mice it was found by *Söremark & Ullberg* (1961) that vanadium showed preference for bones and teeth as a site of accumulation. In the soft tissues surprisingly high concentrations were found in lungs and liver, and in part of the placenta.

In the present paper the distribution of $V_2^{48}O_5$ in developing teeth and bones is studied autoradiographically.

METHODS

The isotopic solution, a carrier-free $V_2^{48}O_5$ solution, was supplied by Philips Duphar, Holland. The radioactive vanadium isotope V^{48} has a half-life of 16.2 days and disintegrates by emission of positrons of 0.69 MeV and three gamma rays of about 1 MeV. Instead of positron emission, electron capture can take place in a small percentage of cases. The shape of the positron spectrum and the relatively hard energies of 0.69 MeV of the beta particles will influence unfavourably the resolution of the autoradiograms. 0.1 ml of physiologic saline solution with 10 μC V^{48} added in the form of carrier-free $V_2^{48}O_5$ solution in minute amounts, was injected subcutaneously in single doses to young rats, 7 and 9 days old. Of the radioactive solution injected 10 μC was equivalent to less than 10^{-9} mg of vanadium.

Two rats were used in the present studies. 24 hours after the injection the animals were rapidly frozen by immersion in a mixture of solid carbon dioxide and acetone (about $-70°$C). The head and a hind limb were cut free and mounted on a microtome stage. These and further procedures were carried out in a freeze room at $-10°$C. Ten microns thick sections were then taken at various levels through the head and femur and were allowed to freeze-dry for 24 hours. Then the sections were dry-mounted on Ilford G5 nuclear emulsions. After exposure for about four weeks the tape was removed by immersion in xylene. The emulsions with the sections in permanent contact were developed and fixed; then the sections were stained in haemalum-eosin and section + emulsion were mounted in Canada balsam. Further details regarding the autoradiographic technique used were described by *Ullberg* (1958).

RESULTS AND DISCUSSION

Generally, the autoradiograms showed that the dentine in the tooth germs took up much more radiovanadium than did the enamel. The distribution in enamel, dentine, and bone varied between different teeth and bones and also between different parts of the same tooth or bone. The greatest uptake of V^{48}, was

found in parts of teeth and bone where rapid mineralization was taking place.

In those teeth which had reached the furthest stages of development it was found that radiovanadium was present in the pulps in very small concentrations and diffusely distributed. Vanadium also appeared in about the same low concentration in the odontoblast zone. In the predentine zone no appreciable uptake of radiovanadium was observed.

In the dentine, V^{48} was diffusely distributed; however, in the part adjacent to the predentine a narrow zone with higher uptake could be found. The resolution of the autoradiograms did not permit studies of the localization of V^{48} in the various dentinal structures.

In the incisor enamel, the main uptake of radiovanadium was found in a zone adjacent to the ameloblast layer. However, in the enamel of the molars V^{48} was more diffusely distributed.

In the bones, an abundant uptake of V^{48} could be seen in the subperiosteal layers as well as in the periphery of the trabeculae. The areas of rapid accretion along the peripheral periosteum showed a high uptake. In the bone shaft, uptake of V^{48} was seen both in the subperiosteal and endosteal layers. A heavy uptake of V^{48} was found in the ossification centers of the epiphyseal cartilage and in the calcifying bone trabeculae below the epiphyseal plate. In the cartilage, the uptake of radiovanadium was very low.

It has been established that vanadium is normally present in trace amounts in teeth and bone; exact data are, however, not available. *Geyer* (1953) found that vanadium probably replaces phosphorus in the apatite crystal. Thus, the radiovanadium injected into the animals probably was exchangeable with a small fraction of the inactive vanadium naturally present in the crystals and probably with a fraction of the phosphorus.

SUMMARY

Radiovanadium in the form of $V_2^{48}O_5$ was injected subcutaneously into rats, 7 and 9 days old. By means of microautoradiography the distribution in developing tooth enamel, dentine, and bone was studied.

The investigation showed a higher uptake of V^{48} in dentine

than in enamel. Within the dentine the highest concentration of V^{48} appeared in a zone adjacent to the predentine. The highest uptake was found in areas of rapid mineralization in dentine and bone. In the enamel, the radiovanadium showed an even distribution with an increase in the concentration adjacent to the ameloblast layer of the incisors.

RÉSUMÉ

LOCALISATION AUTORADIOGRAPHIQUE DE PENTOXYDE DE VANADIUM ($V_2^{48}O_5$) DANS LES DENTS ET OS EN MINÉRALISATION DE RATONS

La distribution de radiovanadium, sous-cutanément injecté, dans les dents, l'émail et les os en développement de ratons âgés de 7 et 9 jours a été étudiée à l'aide de micro-autoradiographie.

L'incorporation de V^{48} était plus grande dans la dentine que dans l'émail. Dans la dentine la plus grande concentration se trouvait dans une zone près de la prédentine, qui n'incorporait pas le radiovanadium. L'incorporation principale fût trouvée dans les zones de dentine et d'os à minéralisation rapide. Dans l'émail le radiovanadium montrait une distribution régulière avec une concentration dans une zone attenante à la couche améloblastique des incisives.

ZUSAMMENFASSUNG

AUTORADIOGRAPHISCHE LOKALISATION VON VANADIUMPENTOXID ($V_2^{48}O_5$) IN SICH ENTWICKELNDEN ZÄHNEN UND KNOCHEN VON RATTEN

Die Verteilung des subkutan als V^{48} injizierten Radiovanadiums in Zahnanlagen, Schmelz und Knochen 7 und 9 Tage alter Ratten ist mit mikroautoradiographischer Technik untersucht worden.

Die Aufnahme von V^{48} war grösser im Dentin als im Schmelz. Im Dentin zeigte sich die grösste Aufnahme von V^{48} in einer Zone nahe dem Prädentin, das Radiovanadium nicht aufnahm. Die hauptsächliche Akkumulation wurde in Dentin- und Knochenzonen mit schneller Mineralisation gefunden. Im Schmelz zeigte das Radiovanadium eine gleichmässige Verteilung mit grösserer Konzentration nahe der Ameloblastenschicht der Nagezähne.

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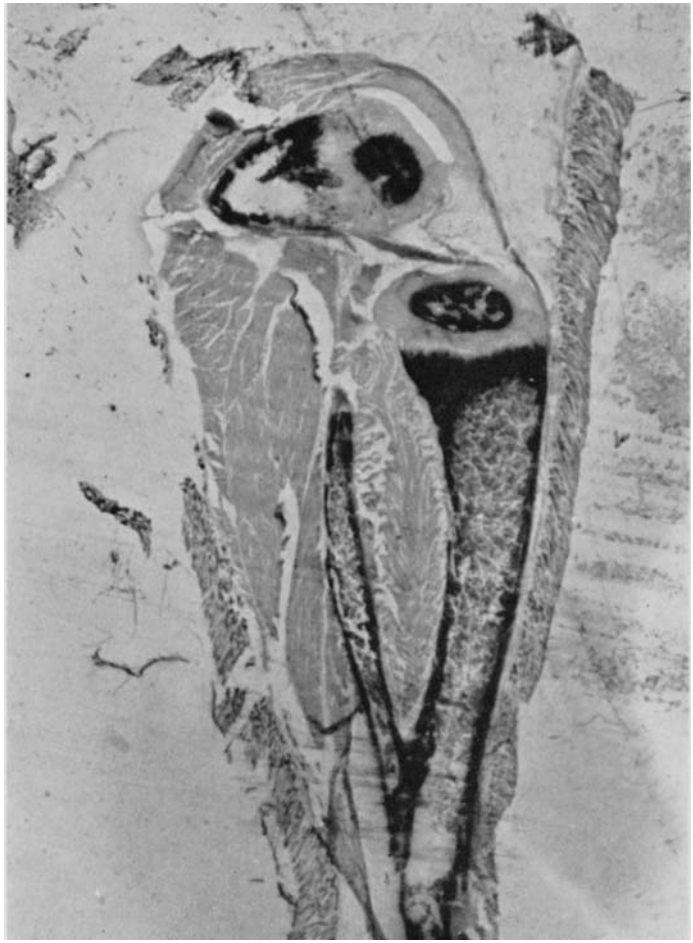

Fig. 1. Autoradiogram showing the uptake of V^{48} in femur, tibia, and fibula of a rat 24 hours after a subcutaneous injection. The section is haemalum-eosin stained and is permanently combined with emulsion. Black dots and areas indicate the uptake of V^{48}. Note high radioactivity in the mataphyseal ossification centers and the subperiosteal zones. Ilford G5, 10 ×.

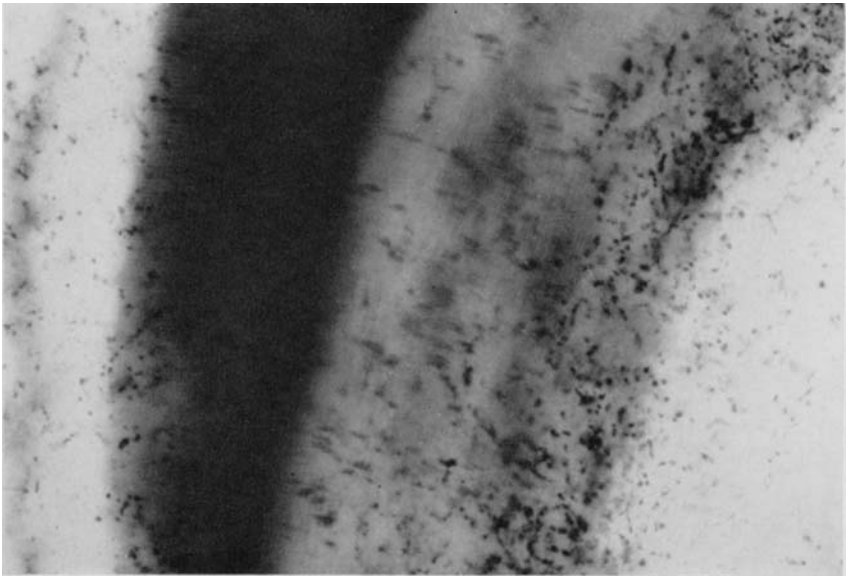

Fig. 2 A

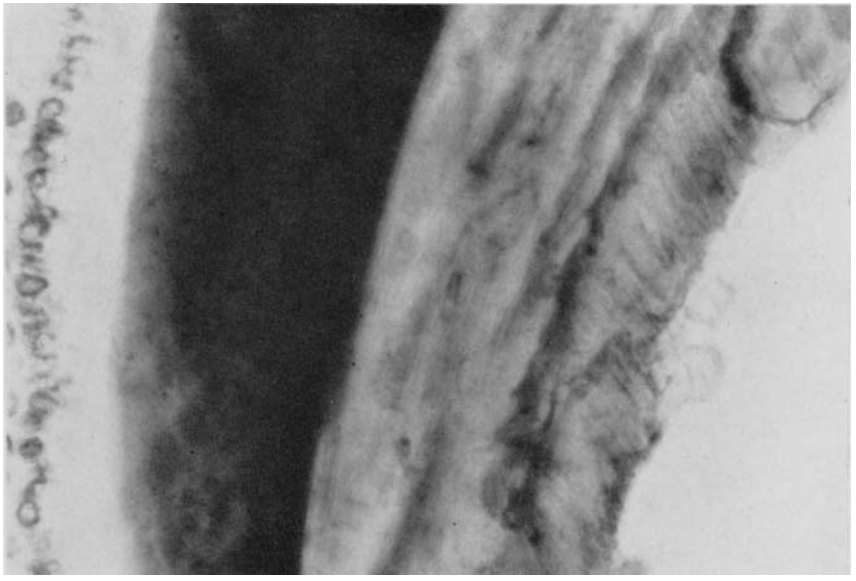

Fig. 2 B

Fig. 2. Part of lower second molar of a 9 day old rat 24 hours after injection of $V_2^{48}O_5$. The focal level of the microscope is in the tissue section (A) and in the emulsion (B). High uptake of V^{48} can be seen in the pulpal zone of the dentine and a lower and more diffuse uptake in the enamel. Ilford G5, 225 ×.

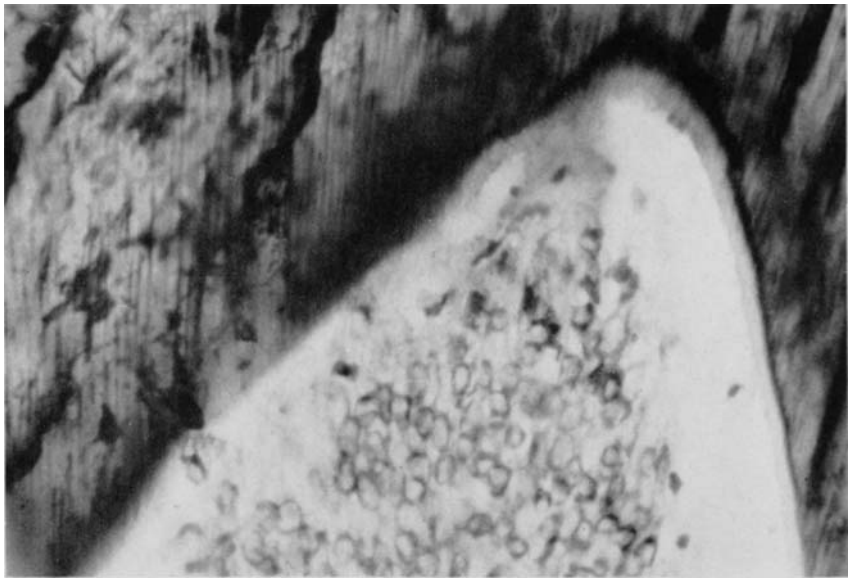

Fig. 3 A

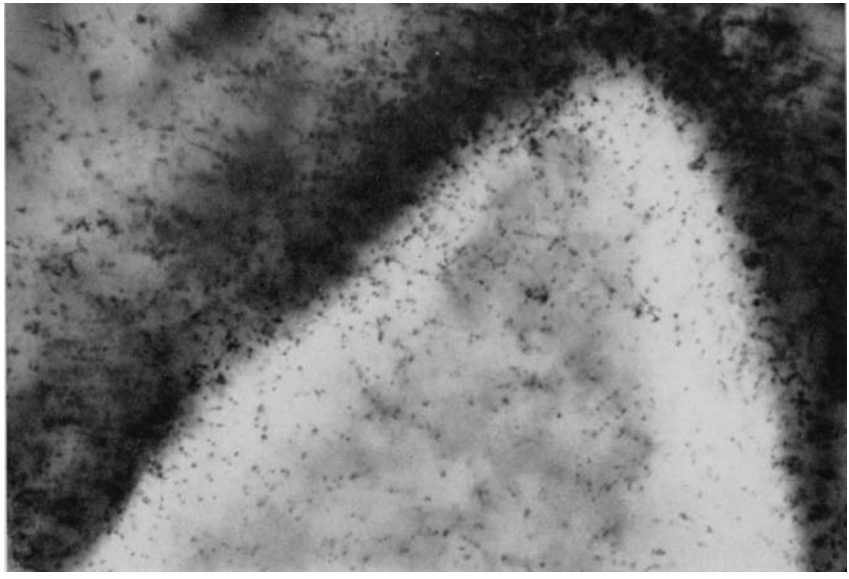

Fig. 3 B

Fig. 3. Part of the occlusal dentinal wall of the pulp cavity from the same tooth as in Fig. 2. The focal level of the microscope is in the tissue section (A) and in the emulsion (B). Especially high uptake is visible in this part of the dentine. Ilford G5, 225 ×.