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UPTAKE AND RELEASE OF VANADIUM FROM INTACT HUMAN ENAMEL FOLLOWING $V_2^{48}O_5$ APPLICATION *IN VITRO*

by

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Attention has been drawn to the fact that vanadium is isomorphous with phosphorus and can replace it in the hydroxy apatite crystals in teeth (cf. the review by *Underwood,* 1956). *Manly & Bibby* (1949) found that some vanadium compounds reduced the acid solubility of enamel more than did a 0.1 % sodium fluoride solution. In the light of this it has been suggested that vanadium present in the hard tissues of teeth has a caries inhibiting effect.

The effectiveness of vanadium as a caries inhibitor has been the object of comparatively few investigations. *Rygh* (1949) and *Geyer* (1953) found that vanadium pentoxide had an inhibiting effect on dental caries in the following experimental animals: rats, Guinea pigs, and Syrian hamsters. *Hein & Wisotzky* (1955), however, studied the effect of a 10 ppm vanadium drinking solution on dental caries in Syrian hamsters and, contrary to the observations reported by *Rygh* and *Geyer,* they did not find any reduction in dental caries. Nor did *Muhler* (1957) note any caries reduction in rats in his studies using 10—40 μg of vanadium pentoxide per ml of drinking water. Recently, *Tank & Storvick* (1960) found a significant decrease in the dental caries rate in permanent teeth of children with increasing vanadium concentrations in the drinking water, ranging from 0.03 to 0.22 ppm. It will be noted that these concentrations were much lower than those used by *Hein & Wisotzky*, and by *Muhler* in their contradictory observations.

Vanadium is said to be normally present in human teeth in minute concentrations (cf. the reviews by *Underwood*, 1956, page 385, and *Orban*, 1957, page 57). On the other hand, *Koch, Smith, Shimp, & Connor* (1956) did not find vanadium in human teeth or bone.

Recently, in an investigation using the radioactive vanadium isotope V^{48} the distribution and kinetics of intravenously administered vanadium pentoxide ($V_2^{48}O_5$) were studied in mice: the radiovanadium was observed to accumulate in bones and teeth in high concentrations. Interesting findings regarding the accumulation of V^{48} in soft tissues were also observed (*Söremark & Ullberg*, 1962). *Söremark, Ullberg, & Appelgren* (1962) studied the uptake of $V_2^{48}O_5$ in developing teeth and bones of rats after an intravenous injection of the radioactive solution. Vanadium seemed to be incorporated into hydroxy apatite crystals during their formation in hard tissues in the same manner as some other elements e.g. phosphorus, fluorine, calcium.

The purpose of the present investigation was to study the uptake and release of vanadium from human enamel after a topical application of V_2O_5 with respect to the possible use of vanadium as a topically applied caries inhibitor. It seemed of importance, therefore, to study the uptake in intact enamel and in enamel with restorations. The radioactive vanadium isotope V^{48} is available carrier-free and has physical properties well suited for studies of the types mentioned.

MATERIAL AND 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.

Ninety-eight intact premolars, extracted for orthodontic reasons from 13- to 16-year-old boys and girls, were used in the present study. Immediately after extraction the teeth were washed in saline solution for one minute and then stored in a refrigerator at 1°C until used, but not longer than for 2 days. When used the teeth were kept at about 20°C. Care was taken not to dehydrate the teeth.

For quantitative measurements of the uptake of V^{48} in the enamel surface the following procedures were used. The teeth were divided into halves, one buccal and one lingual, along a longitudinal plane through the middle of the proximal surfaces. The roots were separated from the crowns at the cemento—enamel junction. The buccal and lingual pieces were covered with wax except for circular areas, 4 mm in diameter, on the buccal and lingual enamel surfaces *ad modum Ericsson* (1958). The tooth pieces, i.e. the uncovered buccal and lingual enamel surfaces, were exposed for varying time periods to a solution consisting of 25 ml physiologic saline solution to which a trace amount of radioactive vanadium pentoxide solution ($V_2^{48}O_5$) had been added. Less than 10^{-5} μg carrier-free radiovanadium was present in the solution; stable vanadium pentoxide was also added until the concentration was 0.1 ppm. The exposure periods of the enamel surface to the radioactive vanadium pentoxide solution can be seen in Fig. 3. After exposure the specimens were rinsed for 1 minute in running tap water and then dried with air-blast. The enamel surfaces to be examined were lightly buffed to remove any surface film and debris. A scintillation detector (P-20 DQG) with a cylindrical crystal [1″ · 1″ NaI(Tl)] was used in combination with a SC 72 scaler *(Tracerlab)*.

The rate of wash-out of V^{48} from the buccal and lingual enamel surfaces which had been previously immersed in the radioactive vanadium solution for 72 hours was studied in the following wash solutions: physiologic saline solution, tap water, 0.05 ppm, 0.1 ppm, and 10 ppm vanadium pentoxide solution, and 10 ppm phosphorus pentoxide solution. The solutions had a pH of about 7. During the wash-out procedure the wash solutions were repeatedly changed.

In order to record the rate of uptake and the rate of release of V^{48} the specimens were immersed in the solutions in question repeatedly throughout the experiment. The crown pieces were fixed on removable mounts in aluminium dishes and so oriented that their position with respect to the crystal of the scintillation counter would be the same each time they were placed beneath the crystal for radioactivity measurement. No self-absorption of the gamma radiation in the enamel was taken into account.

For the autoradiographic study the teeth were used in their entirety. Their apices were sealed with wax. After having been stored in the radioactive solution for 1 hour, 2, 24, 48, and 72 hours the teeth were embedded in a special acrylic resin *(Castolite)*. They were then cut in longitudinal sections about 150 microns thick with a diamond instrument. These sections were placed in contact with *Gevaert's Dentus Rapid X-ray* emulsions for exposure for about one week in order to obtain autoradiograms.

In another series for autoradiographic studies restorative fillings of amalgam, gold, silicate cement, and heat-curing acrylic resin were made in class V cavities on the newly extracted intact teeth in order to study the penetration of radiovanadium along the margin between the tooth and the filling material. No isolation or sealing agents were used in the cavities. The ordinary procedures for processing the filling materials recommended by the manufacturers were followed carefully. While the teeth were being filled and while in the radioactive solution they were kept at about 20°C. In this series the area not covered by wax (i.e. the area to be exposed to the solution) was located only along the occlusal margin of the filling. In three teeth the prepared class V cavities were unfilled and the whole cavity not covered by wax. The teeth were immersed in the radioactive vanadium solution for 2 or 24 hours.

RESULTS

Autoradiographic observations

The autoradiograms showed a heavy uptake of radiovanadium in the whole tooth surface exposed to vanadium pentoxide ($V_2^{48}O_5$) solution. The uptake was more pronounced in the cementum than in the enamel. It appeared, with respect to the insufficient resolution in the autoradiograms obtained with the hard beta rays of V^{48} and the very thick sections, that radio-vanadium was absorbed only at the enamel surface and did not seem to penetrate into the enamel even after an exposure time of 72 hours in the radioactive solution. In the root, on the other hand, radiovanadium seemed to penetrate the cementum and into

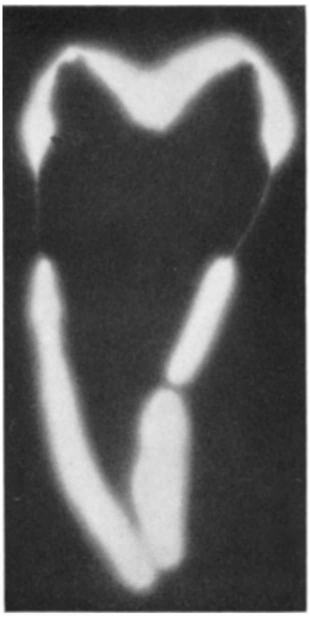
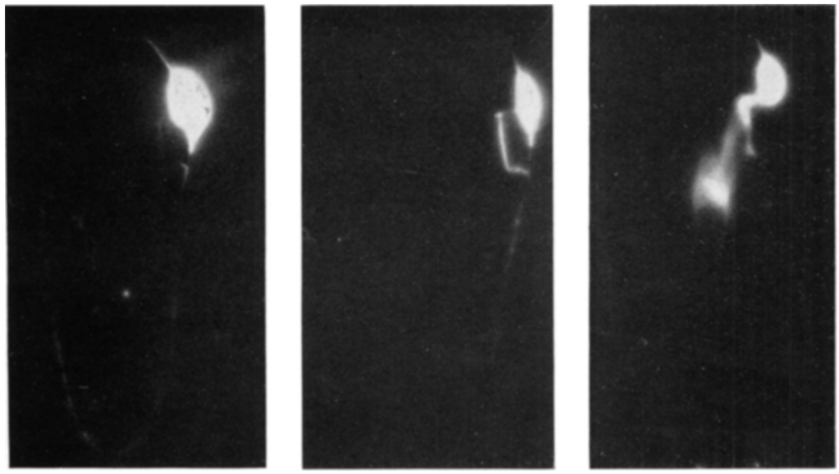


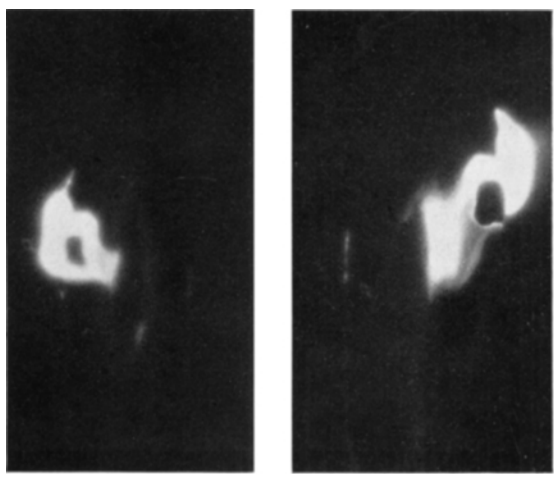
Fig. 1. Autoradiogram showing the uptake of V^{48} in a tooth surface after immersion for 24 hours in a V^{48}-labelled vanadium pentoxide solution. White areas correspond to high uptake of radiovanadium.

the dentine. This was observed in those teeth which had been exposed to the radioactive solution for time periods of 48 hours or longer. In areas of the buccal surface of the crowns where class V cavities had been prepared but not filled a very heavy uptake of V^{48} was observed in the dentine, and radiovanadium penetrated the dentine into the pulp. This was seen after only 2 hours' immersion. It was also found that the innermost layers of the enamel in the cavity wall were more permeable to vanadium than the surface layer.

The teeth in which various filling materials had been inserted showed a more or less pronounced penetration of radiovanadium into the margin between the filling material and the cavity wall



Gold + ph. cement Acrylic + ph. cement Acrylic + acrylic



Silicate Amalgam

Fig. 2. Autoradiograms showing the uptake of V^{48} in the margin between tooth and various types of restorations in the buccal enamel surface after 24 hours' immersion in a radiovanadium-labelled solution. White areas correspond to high uptake of V^{48}.

Thus, in teeth with acrylic resin and amalgam and also silicate fillings the heaviest uptake was found in the enamel and dentinal walls. In these cases radiovanadium penetrated along the dentinal tubules into the pulp after 24 hours' immersion. The acrylic resin fillings were ordinarily polymerized by heating up to 100°C, the fillings then being "cemented" in the cavities with cold-polymerizing acrylic resin. In two cases, where these fillings were fastened with phosphate cement, there was but little penetration into the cement layer. Cemented gold inlays showed the smallest penetration of V^{48} into the enamel-filling margin.

Quantitative measurements of the uptake of V^{48} in enamel

Fig. 3 shows the uptake of V^{48} in the buccal and lingual enamel surfaces repeatedly immersed in the $V_2^{48}O_5$ solution. Each curve represents the mean of about 50 surfaces. There were great differences in the amount of vanadium taken up in the enamel surfaces, but there were always relatively small and non-significant

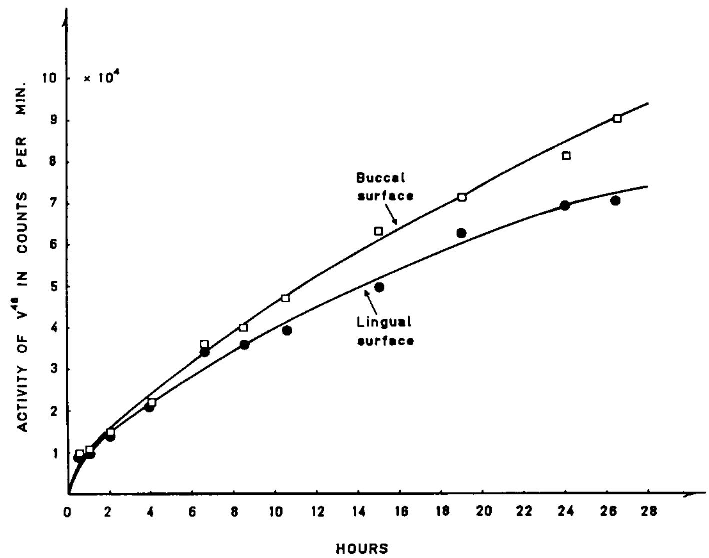


Fig. 3. Rate of V^{48} uptake by intact enamel, buccal and lingual surfaces, immersed in a V^{48}-labelled vanadium pentoxide solution. The curves illustrate the mean uptake of V^{48} of 50 buccal and 48 lingual surfaces repeatedly immersed in the radioactive solution for various periods of time. The lingual surfaces showed a somewhat slower rate of the mean uptake of V^{48} than did the buccal surfaces; this difference was, however, non-significant.

differences between the uptake of buccal and lingual surfaces of the same tooth. No significant difference of the uptake of radiovanadium due to sex was found.

Quantitative measurements of the release of V^{48} from enamel

Figs. 4 and 5 show the rate of wash-out of V^{48} from the enamel surfaces when exposed to tap water, physiologic saline solution, 0.05 ppm, 0.1 ppm, and 10 ppm vanadium pentoxide solution, and a 10 ppm phosphorus pentoxide solution. Each curve repre-

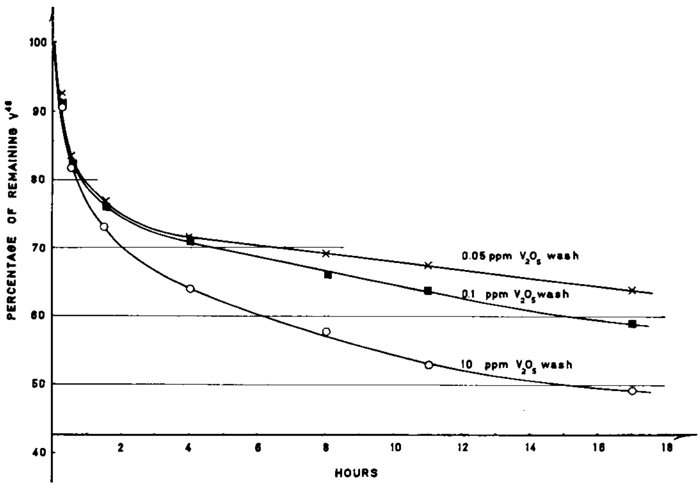


Fig. 4. Rate of release of V^{48} from intact buccal and lingual enamel surfaces by washing in 0.05, 0.1, and 10 ppm stable vanadium pentoxide. Each curve illustrates the mean of 20 enamel surfaces.

sents the mean of 20 enamel surfaces. The original activity of V^{48} in the enamel surfaces was built up by immersion in the radioactive solution for 72 hours. The most pronounced loss of V^{48} from the enamel was from surfaces immersed in a 10 ppm phosphorus pentoxide solution. When the enamel surfaces were washed in 10 ppm vanadium pentoxide solution the rate of release of V^{48} was about the same as when they were washed in 10 ppm phosphorus pentoxide solution. The rate of release of V^{48} when the enamel surfaces were washed in tap water and physiologic

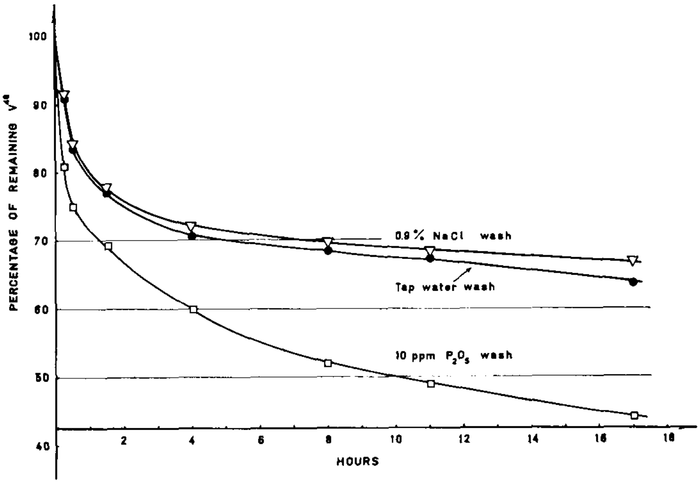


Fig. 5. Rate of release of V^{48} from intact buccal and lingual enamel surfaces by washing in tap water, physiologic saline solution, and 10 ppm phosphorus pentoxide solution. Each curve illustrates the mean of 20 enamel surfaces.

saline solution was rather slow and about the same for both solutions. No significant difference in the rate of release from the buccal and lingual surfaces or due to sex was found.

DISCUSSION

If a simple iso-ionic exchange at the enamel surface occurs, in which the radioactive vanadium of the immersing solution exchanges with stable vanadium normally occurring in the enamel, it probably takes place to a very small extent, because vanadium if present at all occurs in very small amounts. It would seem that rather there is a hetero-ionic exchange, i.e. a substitution of vanadium for phosphorus in the hydroxy apatite. Thus, the rate of release of V^{48} from the intact buccal and lingual enamel surfaces, which had been previously immersed in the radioactive vanadium solution for 72 hours, was found to be about the same when washing in 10 ppm vanadium pentoxide solution and in 10 ppm phosphorus pentoxide solution. In this connection it should be recalled that there is normally an exchange of phosphate ions between enamel and saliva (*Pedersen & Schmidt-Nielsen*, 1942, and *Ericsson*, 1949). In the present study only buccal and lingual surfaces have been used. *Ericsson* (1958) observed that the uptake of P^{32} of proximal surfaces with incipient caries was 6—8 times as great as that of the buccal surfaces. No significant difference, however, was observed in the uptake of P^{32} between buccal and lingual enamel surfaces. This is in agreement with the present observations on radiovanadium.

The rate of release of F^{18} into 1 ppm and 100 ppm NaF solutions was found by *Fremlin, Hardwick, & Mathieson* (1959) to be rather rapid. The rate of release of vanadium, found in the present study, was much slower. It will be noted that also the rate of uptake and the penetration of vanadium into the intact enamel was slower than that of fluoride. Consequently, the exchange of vanadium for phosphorus in the hydroxy apatite crystal of enamel seems to be much slower than that of fluoride for OH^-.

There also seem to exist some close similarities between the findings of the present study and those regarding the permeability of the enamel to fluoride ions (*Ericsson,* 1958, and *Hardwick & Fremlin,* 1959). Thus, in the present study there is evidence suggesting a slower rate of penetration of the outer enamel by vanadium than of the inner parts. This is in accordance with the penetration of fluoride. Teeth with and without fillings in the prepared cavities in the buccal surfaces clearly showed that V^{48} penetrated into the enamel wall even when these teeth were exposed to the radioactive solution for only two hours. In the intact enamel surface, on the other hand, the mean depth of penetration of radiovanadium seemed to be very small even at the longest exposure time of 72 hours. The permeation through the dentine was, however, very rapid.

The present experiments were performed on teeth from boys and girls aged 13 to 16. Therefore, nothing can be said about differences in the uptake and penetration rates due to age. No significant differences were, however, apparent between the indices of penetration in 13- and 16-year-old subjects. Nor were any found due to sex.

The autoradiograms showing the uptake of radiovanadium in the margin between cavity walls and fillings of varying materials may give an idea about the fitting of the fillings and permeability of the filling material cf. *Going, Massler, & Dute* (1960) and *Swartz & Phillips* (1961).

Fremlin, Hardwick, & Mathieson (1959) discussed the value of isotope techniques in studying the uptake and release of small ions by the hard dental tissues in experiments *in vitro*. *Ericsson* (1958) discussed the effect of polishing the enamel surface before topical application.

Our studies of the uptake of vanadium compounds in the hard tissues of teeth are being continued.

SUMMARY

1. The uptake of radiovanadium in intact tooth surfaces was studied by means of autoradiography and scintillation counting methods using V^{48}-labelled vanadium pentoxide solution.
2. The rate of release of radiovanadium from enamel surfaces which had been immersed in a V^{48}-labelled vanadium pentoxide solution was studied by washing the specimens in tap water, physiologic saline solution, 0.05, 0.1, and 10 ppm stable vanadium pentoxide solution and 10 ppm phosphorus pentoxide solution. The results are shown graphically.
3. The uptake of radiovanadium in the margin between the cavity wall and various filling materials was studied autoradiographically.

RÉSUMÉ

L'ABSORPTION ET L'ÉLUTION DE VANADIUM D'ÉMAIL INTACT D'HOMME SUIVANT APPLICATION DE $V_2^{48}O_5$ *IN VITRO*

1. L'absorption de radiovanadium aux surfaces dentaires a été étudiée par autoradiographie et un procédé de comptage de scintillation en employant pentoxyde de vanadium marqué de V^{48}.
2. Le degré d'élution de radiovanadium de surfaces d'émail immergés dans une solution de pentoxyde de vanadium marqué de V^{48} a été examiné en rinçant les spécimens en eau de la distribution, en solution de sel physiologique, et en solutions de pentoxyde de vanadium stable aux concentrations de 0,05, 0,1 et 10 ppm, et en solution de 10 ppm de pentoxyde de phosphore. Les résultats ont été marqués graphiquement.
3. L'absorption de radiovanadium à la marge entre la dent et les différentes matières de plomb a été examinée par autoradiographie.

ZUSAMMENFASSUNG

AUFNAHME UND AUSSCHEIDUNG VON VANADIUM VON INTAKTEM MENSCHLICHEM SCHMELZ NACH APPLIKATION VON $V_2^{48}O_5$ *IN VITRO*

1. Unter Benutzung von $V_2^{48}O_5$ wurde die Aufnahme von radioaktivem Vanadiumpentoxyd durch intakte Zahnoberflächen mit Hilfe autoradiographischer und Scintillator-Rechnungsmethoden untersucht.

2. Das Ausmass der Ausscheidung des radioaktiven Vanadiums von Schmelzoberflächen, die in $V_2^{48}O_5$ Lösung gelegt worden waren, wurde untersucht mittels Waschung der Versuchskörper in Leitungswasser, physiologischer Kochsalzlösung 0,05, 0,1 und 10 ppm stabiler Vanadiumpentoxydlösung und 10 ppm Phosphorpentoxydlösung. Die Ergebnisse wurden graphisch registriert.

3. Die Aufnahme von radioaktivem Vanadium in der Grenzschicht zwischen Schmelz, Dentin und den verschiedenen Füllungsmaterialien wurde autoradiographisch untersucht.

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