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THE DISTRIBUTION OF SIMULTANEOUSLY ADMINISTERED FLUORIDE AND MOLYBDATE STUDIED WITH F^{18} AND Mo^{99} IN THE RAT

by

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INTRODUCTION

Of potentially caries-inhibiting trace elements other than fluorine, molybdenum is probably the one that has attracted greatest interest. The reports on its effects are, however, conflicting. Some evidence points towards an enhancing effect of molybdenum on the utilization of ingested fluoride, but published reports vary in this respect also. Previous investigations on molybdenum, caries and fluoride have been reviewed by *Shaw & Griffiths* (1961) and by *Büttner* (1961, 1963); these articles also contain the results of original investigations. There are also later papers indicating a caries-preventive action of molybdenum in rat experiments or in the human (*Ludwig et al.*, 1963, *Ludwig,* 1963, *Malthus et al.*, 1964, *Anderson,* 1965).

From studies in other fields molybdenum is known to be of biological significance in several other connections. It is an essential nutrient for all nitrogen-fixing microorganisms and hence of agricultural interest especially for the growth of legumes. On the other hand excessive amounts of molybdenum cause a disease in cattle characterized by drastic diarrhoeas. Interactions occur between molybdenum, copper and inorganic sulphate; molybdenum and copper are antidotes of each other. Tungsten is also a molybdenum antagonist.

Molybdenum has further been found to be contained in the flavoprotein enzymes, xanthine oxidase and nitrate reductase, and the possible essentiality of molybdenum from this point of view

has been extensively studied. There is considerable evidence that molybdenum is essential for the purine metabolism and normal growth of chicks, while rats and other mammals seem to be less affected by molybdenum depletion.

Previous work on molybdenum metabolism has given no clear indication of the mechanism of molybdenum interference with the caries process, if any.

The investigation to be presented was designed to compare the distribution in some of the rat organs of F^{18}-labelled fluoride and Mo^{99}-labelled molybdate following peroral ingestion, and the possible influence of the molybdate on the fluoride distribution.

MATERIAL AND METHODS

Fasting male Sprague-Dawley rats, of standardized stock and breeding and weighing about 200 g, were fed 1-mM F^{18}-labelled sodium fluoride and 0—0.5-mM sodium molybdate in 7 ml milk by stomach tube. (The fluorine and molybdenum contents of ordinary cow's milk are about 0.01-mM and 0.005-mM, respectively.) In some of the experiments the molybdate was labelled with Mo^{99}. Fluoride and molybdate were added to the milk as concentrated neutral water solutions, which diluted the milk by 10 per cent.

Groups of four rats each were sacrificed after one and four hours, with or without previous collection of saliva under nembutal anaesthesia and pilocarpine stimulation. Samples of heart blood were taken in ether anaesthesia and after sacrifice by overstretching the spine samples of the liver, one kidney, one femur and the whole digestive tract were removed for analysis.

F^{18} decays with a half-life of about 110 min, while the half-life of Mo^{99} is about 67 hours. A double assay procedure, immediately after the experiment and again after the complete decay of F^{18}, thus provides a method of separating the activities of the two isotopes. The gamma radiation accompanying the beta decay of both isotopes is most convenient for the measurement.

Scintillation counting in a well-type crystal was used for the assay of the isotopes and an electronic computer programme for the calculation of the double-tracer experiments. These methods as well as the production of F^{18} have been described in detail

previously (*Ericsson*, 1958, *Ericsson, Santesson & Ullberg*, 1961, *Ericsson & Hammarström*, 1964). Mo^{99} was produced in the neutron reactor R 2 of Atomenergi Co. by irradiating MoO_3 in quartz ampoules. For the production of sodium molybdate the irradiated salt was dissolved in a calculated quantity of sodium hydroxide solution. In some of the tests inactive sodium molybdate was added in order to obtain the desired concentration.

In order to compensate for differences in weight of individual animals the activity of each organ except the digestive tract was calculated as the percentage of the dose given per gram animal that was found per gram organ or fluid.

RESULTS

The results of the one-hour tests are given in Figs. 1 and 2. Fig. 1 gives the results including analyses of the saliva, thus obtained with anaesthesia and pilocarpine stimulation. The follow-

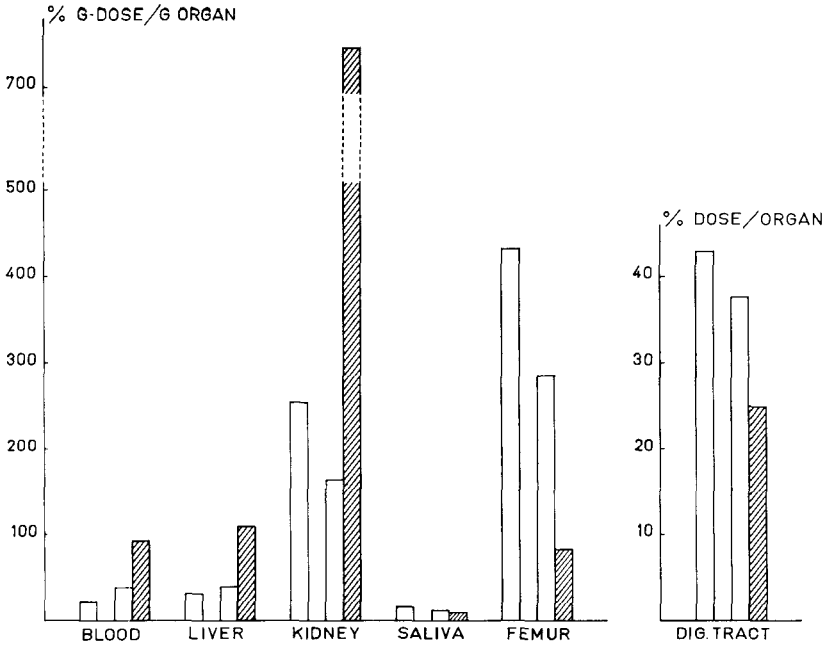

Fig. 1. Distribution of F^{18} separately (▭ , left in each group of 3 columns) and F^{18} + Mo^{99} (▨ , right) one hour after ingestion of 1-mM NaF, with of without 0.5-mM labelled Na_2MoO_4, in 7 ml milk.

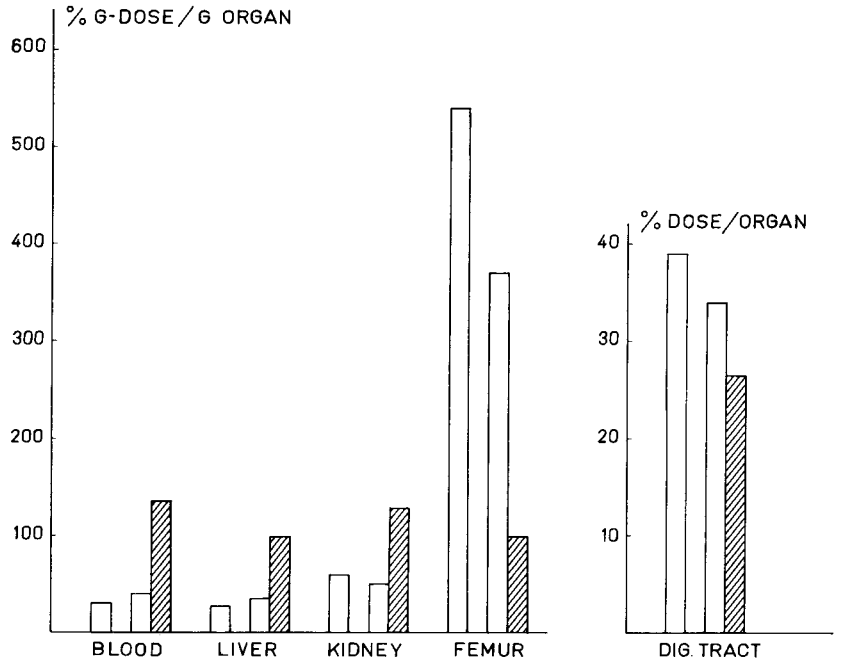

Fig. 2. Result of same experiment as Fig. 1 without collection of saliva. Denotations as Fig. 1.

ing features appear from the diagram and were borne out by the statistical analysis.

The molybdenum was more rapidly absorbed in the intestinal tract than the fluoride, gave higher concentrations in the blood and liver, lower concentrations in the bone, and a rapid excretion (or accumulation) in the kidney.

The fluoride absorption was not significantly changed in the double-tracer test, nor the liver and kidney concentrations, while the blood concentration of fluoride was significantly increased. There was a considerable decrease in the bone uptake of fluoride in the presence of labelled molybdenum.

The salivary excretion of both elements was low, and the presence of molybdenum did not change the salivary fluoride significantly.

The corresponding experiment without collection of saliva and

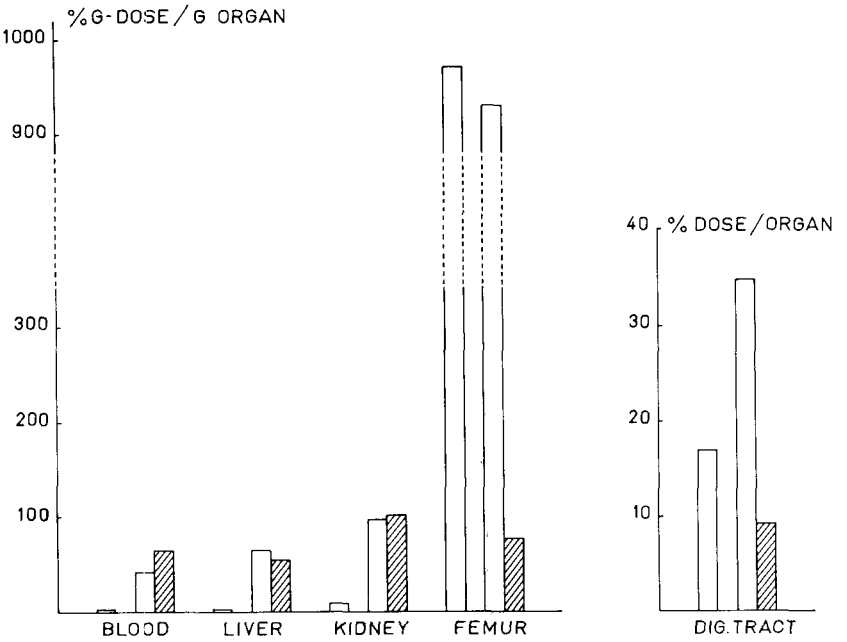

Fig. 3. Distribution of F^{18} separately and $F^{18} + Mo^{99}$ four hours after ingestion of 1-mM NaF, with or without 0.5-mM Na_2MoO_4, in 7 ml milk.

Denotations as Fig. 1.

thus without anaesthesia and pilocarpine injection was carried out as a control of the possibility that nembutal and/or pilocarpine might appreciably change the metabolic pattern. The result of this control test appears from Fig. 2.

The strikingly lower kidney concentrations in this series are evidently due to the absence of pilocarpine. In other respects the results are comparable to those obtained after injection of the drugs except that the liver concentration of F^{18} was significantly increased as well as that of the blood.

After the experimental series with one hour survival time the same technique was tried with four hours survival time. This, however, proved partly impractical since the rats given molybdenum and fluoride plus nembutal and pilocarpine died within four hours. Although the applied molybdenum concentration was only about one per cent of the dietary concentration that is reported as being acutely toxic to rats under normal circumstances,

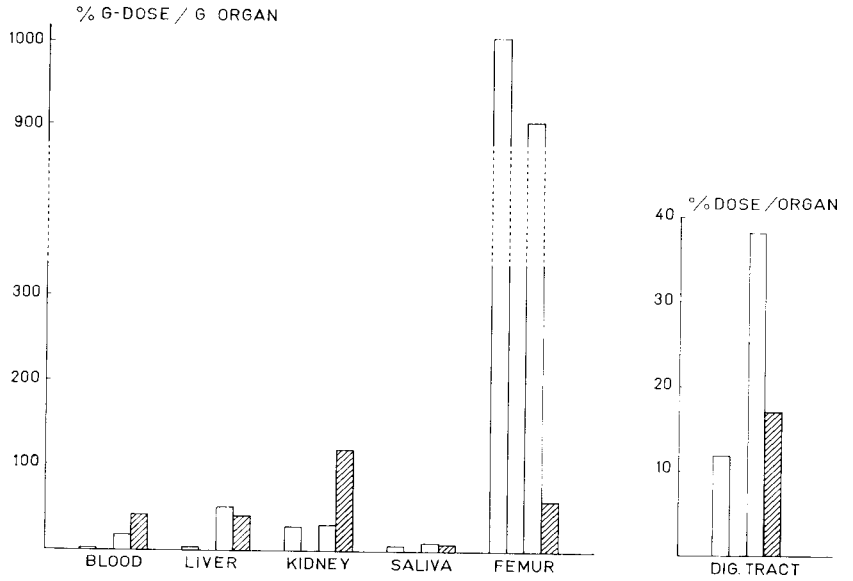

Fig. 4. Distribution of F^{18} separately and $F^{18} + Mo^{99}$ four hours after ingestion of 1-mM NaF with or without 0.05-mM Na_2MoO_4, in 7 ml milk.

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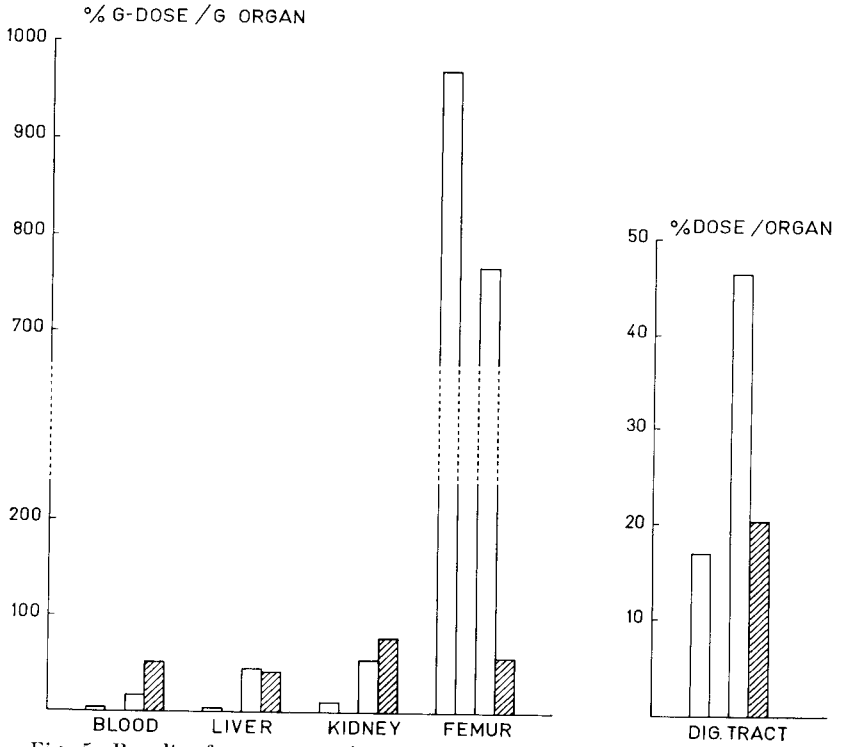

Fig. 5. Result of same experiment as Fig. 4 without collection of saliva.

Denotations as Fig. 1.

the combination with fluoride, nembutal and pilocarpine apparently had a toxic effect after some hours, which was not observed after one hour.

The four-hour test with the 1-mM fluoride and 0.5-mM molybdate concentrations therefore do not include salivary excretion figures. The results of these tests appear from Fig. 3. The rats that were given only fluoride in this case comprised two groups of four each.

After four hours the F^{18} concentration in the digestive tract was about doubled in the presence of labelled molybdenum, and the F^{18} concentrations in the blood, liver and kidney were several times higher in the double-tracer tests. There was no significant difference in the F^{18} uptake of the bones with and without simultaneous molybdenum administration.

A four-hour test series with unchanged fluoride concentration but with the molybdenum concentration reduced to 0.05-mM, i.e. to one-tenth of that used in the previous experiments, was performed without any toxic symptoms of the rats. The control rats given only fluoride were the same as were compared with the four-hour animals given the higher molybdenum dose. The results appear from Figs. 4 and 5.

These tests gave essentially the same results as those given in Fig. 3. Even with this low molybdenum concentration, the intestinal fluoride content was greatly increased after four hours. The bone uptake was slightly decreased while the change in salivary excretion of F^{18} was insignificant.

In the four-hour tests the Mo^{99} concentrations of the different specimens (even the femurs) were lower throughout than those of the one-hour samples.

The fatal results obtained with 0.5-mM labelled molybdenum in combination with pilocarpine and nembutal, and the fact that the neutron-irradiated MoO_3 powder had changed its colour from white to blue caused the suspicion that the irradiation with the accompanying heating of MoO_3 changed this substance in a physiologically important way. Experiments were therefore also carried out with unlabelled sodium molybdate. Fig. 6 shows the results obtained in an experiment with four hours survival time where the effects of both labelled and inactive 0.05-mM molybdate were studied.

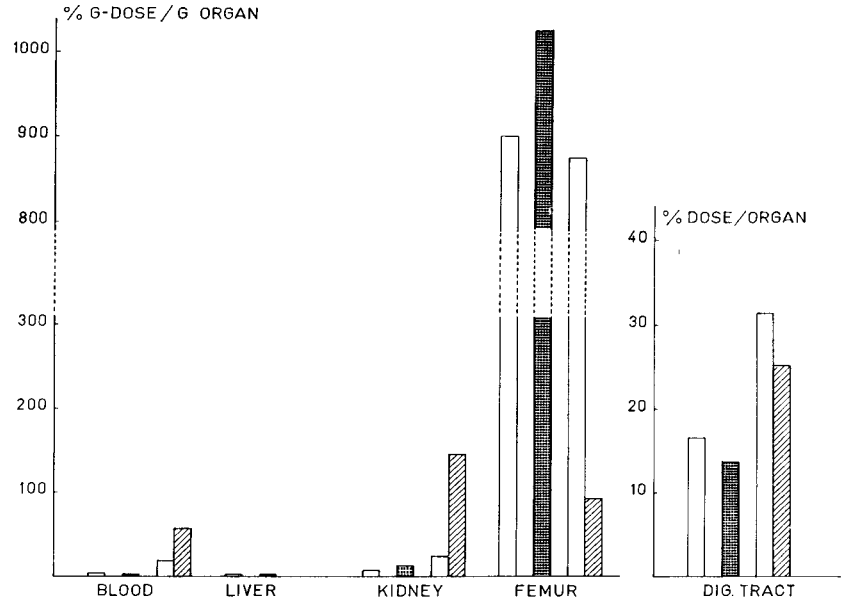

Fig. 6. Distribution of F^{18} separately (▭ , left column in each group), F^{18} with 0.05-mM inactive molybdate (▦) and F^{18} + Mo^{99} as 0.05-mM labelled molybdate (▨ , right pair of columns) four hours after ingestion.

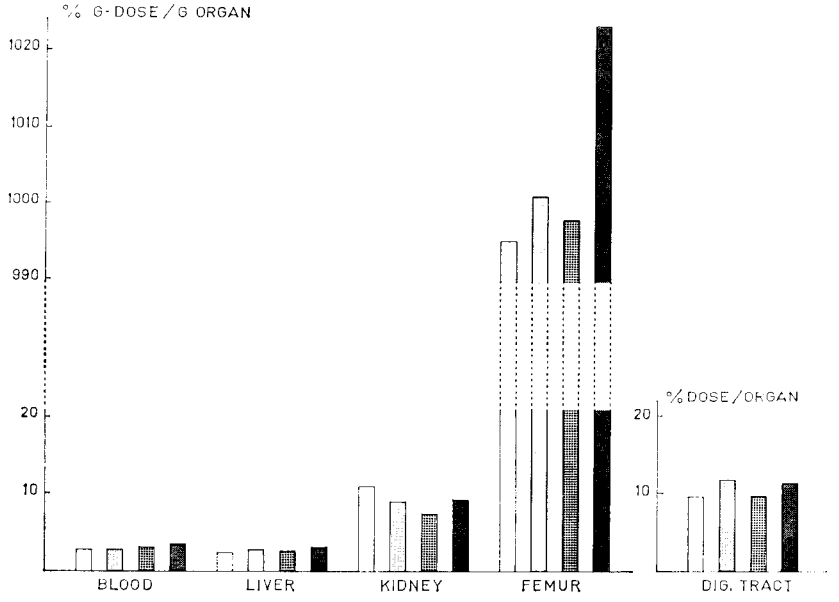

Fig. 7. Distribution of F^{18} with (from left to right in each group of columns) 0-, 0.01-, 0.05- and 0.5-mM unlabelled molybdate.

The molybdate distribution agrees well with that found in the corresponding experiment in Fig. 5. The fluoride concentrations found in blood, kidney and digestive tract in the Mo^{99} experiment were also changed in the same direction, although less than seen in Fig. 5. The femoral uptake was not significantly changed; the liver specimens were damaged and excluded.

However, with inactive molybdate the changes in fluoride distribution were much smaller or even went in the opposite direction.

An experiment was finally performed where the distribution of F^{18}-labelled fluoride was studied in the presence of 0-, 0.01-, 0.05- and 0.5-mM unlabelled sodium molybdate; survival time four hours.

The result appears from Fig. 7. The differences in F^{18} distribution were statistically insignificant throughout, even the apparent femoral increase with 0.5-mM Mo.

DISCUSSION

The molybdenum distribution found in these experiments does not seem to give any indications for a direct effect of molybdenum on the caries process: the molybdenum uptake by the hard tissues (represented by the femur) is comparatively low, and the salivary secretion of molybdenum is also low. Other routes or mechanisms by which molybdenum could theoretically counteract the caries attack are not elucidated by the reported experiments.

If molybdenum had increased the fluoride uptake in the hard tissues or its excretion with the saliva, then this would have indicated mechanisms by which molybdenum could increase the effect of simultaneously ingested fluoride. However, no such effect of molybdenum on the fluoride distribution was found. As regards the absence of any molybdenum effect on the femoral fluoride uptake our findings support those of *Büttner* (1963).

Sodium molybdate prepared from ordinary MoO_3 had no influence on any of the tested parameters of fluoride metabolism. The difference in this respect found with irradiated MoO_3 is enigmatic, at least to the knowledge of the author.

The most remarkable effects of irradiated molybdenum were the strongly increased fluoride activities that were found in the digestive tract and in blood, liver and kidney after four hours. These may be related to the high Mo^{99} concentrations that are found autoradiographically in the walls of the mouse stomach and small intestine (*Ericsson & Söremark,* unpublished data). An increased secretion of the glands of these organs is apparent and may have the same basic mechanism as the diarrhoea that is a clinical characteristic of molybdenum poisoning. These phenomena warrant further study; however, their significance in the caries process is doubtful.

Another finding in the abovementioned autoradiographic Mo^{99} studies of Ericsson and Söremark, which might indicate a caries-preventive mechanism of molybdenum, is a concentration of the isotope in some uterine and foetal structures. This finding may have some connection with *Büttner*'s report (1963) that Mo+F had a caries-reducing effect in the rat when given both pre- and postnatally but not with only postnatal administration, and also with the report of *Healy & Ludwig* (1963) that children in an area with a higher molybdenum supply had an increased Mo content in their deciduous teeth but not in their permanent teeth. This will be the point of departure of a special study.

SUMMARY

The investigation was designed to compare the distribution in the rat of F^{18}-labelled fluoride and Mo^{99}-labelled molybdate following peroral ingestion, and to search for possible caries-preventive mechanisms of molybdenum, e.g. through an influence on the metabolism of fluoride.

Fasting male rats were fed 1-mM F^{18}-labelled sodium fluoride and 0—0.5-mM sodium molybdate in milk by stomach tube; the molybdate was either Mo^{99}-labelled or unlabelled. Groups of rats were sacrificed after one and four hours, with or without previous collection of saliva under nembutal anaesthesia and pilocarpine stimulation. Samples of blood and liver, one kidney, one femur and the whole digestive tract were taken for analysis.

Labelled molybdate greatly increased the F^{18} activities found in blood, liver, kidney and digestive tract after four hours, while

the femoral uptake was either unchanged or decreased after both one and four hours. No such changes were found with inactive molybdate. The explanation of those differences may be connected with changes observed in the MoO_3 used for preparing the molybdate, when the oxide was neutron irradiated.

The Mo^{99} distribution did not give any indications for a direct effect of molybdenum on the caries process. No other findings indicative of a caries-preventive mechanism of postnatally ingested molybdenum were made. However, data are combined in the discussion which support the possibility of a prenatal effect.

ACKNOWLEDGEMENT

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RÉSUMÉ

LA RÉPARTITION DE FLUORURE ET DE MOLYBDATE SIMULTANÉMENT INGÉRÈS, ÉTUDIES DANS LE RAT AVEC F^{18} ET Mo^{99}

Ce travail a eu pour but de comparer la répartition dans le rat de fluorure marqué de F^{18} et de molybdate marqué de Mo^{99} après ingestion perorale, et de rechercher des mécanismes anti-cariogènes éventuels du molybdène, par exemple par une influence sur le métabolisme du fluorure.

Du lait contenant 1-mM de fluorure de sodium marqué de F^{18} et 0—0,5-mM de molybdate de sodium fut administré à des rats mâles à jeun; dans une partie des expériences le molybdate était marqué de Mo^{99}. Des groupes de rats furent sacrifiés au bout d'une heure et au bout de quatre heures, avec ou sans recueil préalable de salive sous anesthésie au nembutal et stimulation par la pilocarpine. Des épreuves de sang et de foie, un rein, un fémur et le tube digestif entier furent prélevés pour être analysés.

Le molybdate marqué augmentait fortement les activites de F^{18} trouvées dans le sang, le foie, le rein et le tube digestif après une heure qu'après quatre heures, tandis que l'activité du fémur était sans changement ou diminuée tant après une heure qu'après quatre heures. Aucun changement comparable ne fut trouvé avec le molybdate inactif. L'explication de ces différences pourrait

avoir rapport à des changements observés après l'irradition neutronique du MoO_3 qui était employée pour la préparation du molybdate.

La répartition du Mo^{99} n'indiquait aucun effet direct du molybdène sur le processus de la carie. Aucune autre observation n'indiquait un mécanisme anti-cariogène de molybdène ingéré après la naissance. Cependant, dans la discussion, on recueille des rapports qui soutiennent la possibilité d'un effet prénatal.

ZUSAMMENFASSUNG

DIE VERTEILUNG VON GLEICHZEITIG ZUGEFÜHRTEN FLUORID UND MOLYBDAT GEMÄSS F^{18}- UND Mo^{99}- STUDIEN AN DER RATTE

Die Untersuchung hatte den Zweck, die Verteilung von F^{18}-markiertem Fluorid und Mo^{99}-markiertem Molybdat in der Ratte nach peroraler Zufuhr zu vergleichen, und nach eventuellen kariesvorbeugenden Wirkungsweisen des Molybdäns zu suchen, z.B. durch einen Einfluss auf den Fluormetabolismus.

Männliche Ratten erhielten nüchtern 1-mM F^{18}-markiertes Natriumfluorid und 0—0,5-mM Natriummolybdat in Milch durch Magenschlauch; das Molybdat war entweder Mo^{99}-markiert oder unmarkiert. Gruppen der Ratten wurden nach einer bzw. vier Stunden geopfert, mit oder ohne vorhergehendes Speichelsammeln unter Nembutalanästhesie und Pilocarpinstimulation. Proben von Blut und Nieren, ein Femur und der ganze Verdauungskanal wurden zur Analyse genommen.

Markiertes Molybdat erhöhte stark die F^{18}-Aktivitäten, die nach vier Stunden in Blut, Leber, Niere und Verdauungstrakt gefunden wurden, während die Aufnahme im Femur entweder unverändert oder vermindert war nach sowohl einer als vier Stunden. Keine solche Einflüsse wurden von inaktivem Molybdat gefunden. Die Erklärung dieser Unterschiede steht möglicherweise im Zusammenhang mit Veränderungen, die nach Neutronenbestrahlung des MoO_3 beobachtet wurden, das zur Bereitung des markierten Molybdates benutzt wurde.

Die Mo^{99}-Verteilung gab keine Andeutung einer direkten Molybdänwirkung auf den Kariesprozess. Keine andere Beobachtungen deuteten einen kariesvorbeugenden Mechanismus postna-

tal zugeführten Molybdäns an. In der Diskussion werden aber Daten zusammengestellt, die die Möglichkeit einer pränatalen Wirkung stützen.

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