From: The Department of Cariology and the Chemical Laboratory, School of Dentistry, Karolinska Institutet, Stockholm, Sweden

BLOOD FLUORIDE CLEARANCE IN RATS DIFFERING IN AGE OR PREVIOUS FLUORIDE EXPOSURE

INVESTIGATIONS USING RADIOACTIVE FLUORINE

by

YNGVE ERICSSON

INTRODUCTION

Particularly with regard to naturally occurring high fluoride concentrations in drinking water it is of importance to clarify as thoroughly as possible the pathways and concentration gradients of absorbed fluoride in different tissues and fluids of the body. This has already been fairly well done as regards bones and urine, the main fluoride "outlets", but less so as regards body fluids other than urine and soft tissues, where the fluoride concentrations are normally very low. Blood is naturally the most important of these fluids.

Smith & Gardner (1956) found that the ingestion by rats of large doses of fluoride caused a rise in the blood concentration that reached a maximum after one-half to one hour. *Armstrong & Singer* (1959) found no elevated plasma fluoride values in human subjects living in areas with up to 2.5 ppm F in the drinking water while persons in an area with 5.4 ppm water fluoride showed clearly increased plasma fluoride values. The time of sampling in relation to food or water intake was not given in this report. *Ericsson* (1958) found distinct F^{18} peaks in the blood of rats following the ingestion with water or milk of 1.2 ppm fluoride as NaF labelled with F^{18}. The blood concentrations of absorbed inactive fluoride that could be calculated from the F^{18} activities were very low, however, and would have been difficult

to distinguish chemically, especially since absorbed fluoride must be assumed to replace part of the blood fluoride which is excreted or sequestered by the skeleton.

In experiments with nephrectomised dogs *Carlson et al.* (1960) were able to demonstrate a notable fluoride homeostasis of the blood entirely effected by skeletal sequestration. In rats raised on widely different fluoride content in food and water *Armstrong & Singer* (1963) found about 50 times more fluoride in the bones of the rats given the high-fluoride ration but still very little elevation of the plasma fluoride of these rats.

There still exists a possibility that a high degree of fluoride saturation of the skeleton, for example in older individuals, may reduce the sequestration rate to such an extent that the blood fluoride may be temporarily more elevated in these individuals. Experiments with previously fluoride-exposed rats by *Stookey, Crane & Muhler* (1964) have indicated a decreased intestinal absorption of fluoride in rats with a high skeletal fluoride content, which might be mediated by changes in the blood fluoride content.

In this investigation F^{18}-labelling of ingested fluoride has been utilized in an effort to throw some additional light on these phenomena and problems.

MATERIAL AND METHODS

For the experiments were used male Sprague-Dawley rats of standardized stock and breeding. Comparisons were made between animals of widely different age and weight, raised by Anticimex Co., Stockholm, or rats of the same age and average weight but kept on drinking water with widely different fluoride content; the latter animals were raised at the Department of Medical Chemistry, University of Göteborg. The Anticimex rats were given a solid food containing about 28 ppm F and tap water containing about 0.2 ppm F. The Göteborg rats were kept on a low-fluoride ration, about 4.6 ppm, and either distilled water or water containing 50 ppm F as NaF.

After fasting overnight, the animals were given milk by stomach tube, with the addition of 1 ppm fluorine as F^{18}-labelled NaF, either the same dose in both animal groups to be compared or

a dose in approximate proportion to the body weight. After different time intervals blood was drawn from the heart, and in some of the tests the digestive tract and one or both femurs were also removed for analysis.

In one experiment F^{18} in saline with a low content of carrier-NaF was injected intravenously on rats of different weights before drawing blood from the heart.

The techniques of F^{18} production and labelling, ingestion, sampling and analysis were largely the same as described previously (*Ericsson*, 1958, *Ericsson, Santesson & Ullberg*, 1961, *Ericsson & Hammarström*, 1964). Blood data were calculated in the first rank as the concentration of absorbed, F^{18}-labelled fluoride per gram blood. Intestinal activities were calculated as per cent of total dose remaining in the whole digestive tract. Femoral activities were calculated as per cent of the dose given per gram animal that was found per gram femur; this makes allowance for individual differences in weight.

Chemically analyzed fluoride in the femurs was related to the dry weight.

The different experiments were carried out as follows.

1. Rats of different age and weight, similar diet, peroral ingestion of similar dose. Pilot study.

Two groups of Anticimex rats weighing about 80 and 320 g, respectively, were given the same dose by stomach tube: 5 ml labelled fluoridated milk followed by 2 ml unlabelled fluoridated milk for rinsing down the activity in the tube. Blood was drawn from two rats of each group after 15, 26, 60 and 120 minutes, respectively (26 mins. instead of intended 30 mins. owing to an initial mistake in the timing).

2. Rats of different age and weight, similar diet, peroral. ingestion of dose in approximate proportion to weight.

The Anticimex rats weighed on an average 74 and 332 g, respectively. The young rats were given 1 + 1 ml milk in the same way as in experiment 1. The older rats were similarly given 4 + 4 ml.

Blood was drawn from four rats of each group after 15, 30, 60 and 120 minutes, respectively.

3. Rats of different age and weight, similar diet, i.v. injection of dose in approximate proportion to weight.

This experiment was carried out in order to study the blood fluoride clearance irrespective of absorption. Anticimex rats weighing about 75 and 300 g respectively, were used for the tests. In a tail vein F^{18}-labelled NaF solution in saline containing 0.5 ppm F was injected during half a minute. The dose was 1 ml for the large rats, 0.25 ml for the small ones. After 5, 10 and 20 mins. survival time, measured from the start of the injection, blood was drawn from the heart of three animals in each weight group.

4. Rats of similar age and weight but reared on widely different water fluoride content, peroral ingestion of similar dose.

This experiment was performed in order to study, in otherwise comparable rats, the possible effect of a greater skeletal saturation of fluoride.

Male Göteborg rats that had been kept on the standardized low-fluoride diet since weaning were used for the tests, which were carried out at the age of 50 days in one series and 82 days in another. In both age groups half of the rats had been kept on the drinking water containing 50 ppm F as NaF, the other half on distilled water, both ad libitum. The younger rats weighed about 150 g at the time of the F^{18} tests, the older rats a little over 300 g.

Each rat was given 5 + 2 ml of the labelled and unlabelled fluoridated milk, respectively. Blood was drawn from the heart after 15, 30, 60 or 120 mins. Immediately thereafter, each animal was sacrificed and the digestive tract and one femur were removed for F^{18} analysis, the other femur for chemical fluoride analysis.

RESULTS

1. Rats of different age and weight, similar diet, peroral ingestion of similar dose. Pilot study.

The results are given by Fig. 1. The younger rats had a higher percentage of the total dose per gram blood. Since all rats obtained the same quantity of labelled fluoridated milk, Fig. 1 also

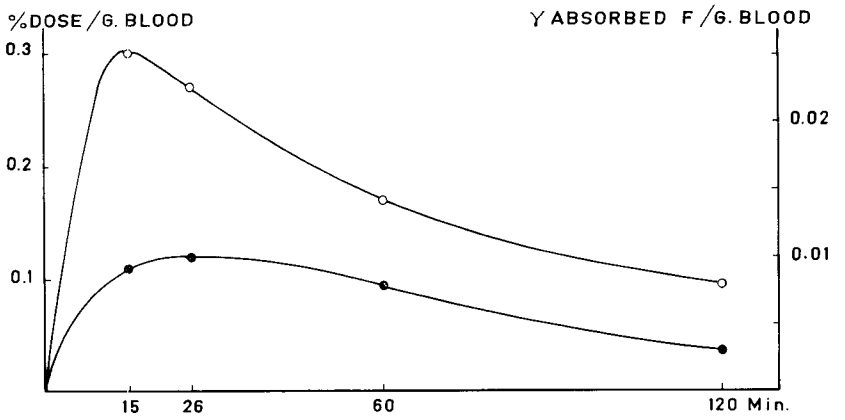

Fig. 1. Blood concentrations of absorbed fluoride following ingestion of similar dose.

o young rats ● old rats

gives the total amounts of absorbed fluoride per gram blood (right ordinate). There was good agreement between duplicate test rats and no overlapping between the groups.

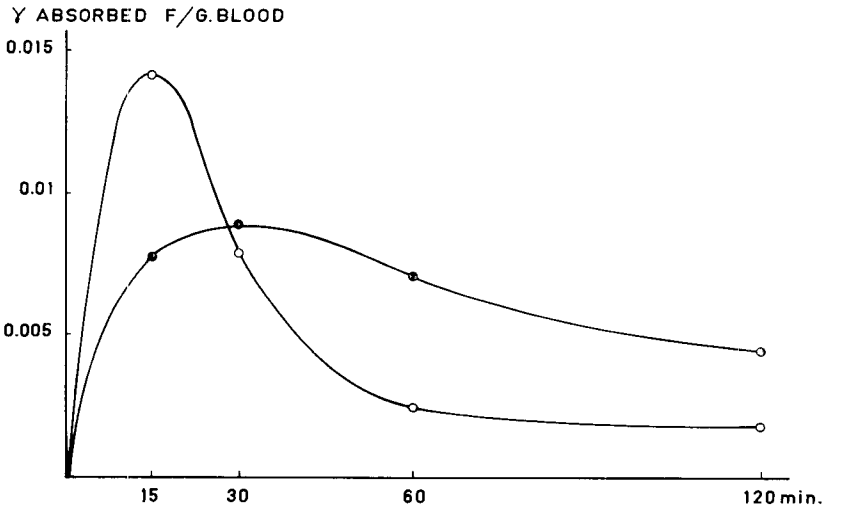

Fig. 2. Blood concentrations of absorbed fluoride following ingestion of dose in approximate proportion to animal weight.

Denotations as Fig. 1.

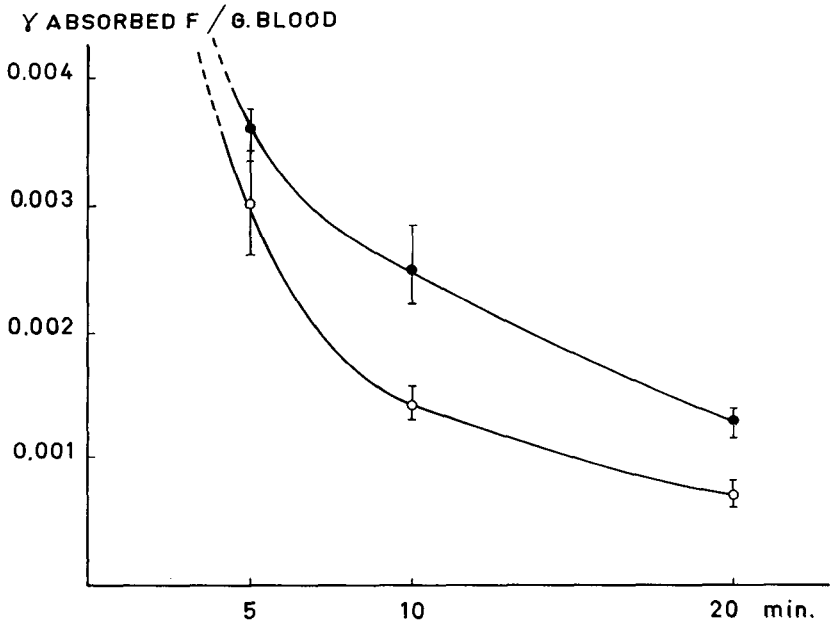

Fig. 3. Blood concentrations of labelled fluoride following i. v. injection of dose in approximate proportion to animal weight. (Averages and ranges.) Denotations as Fig. 1.

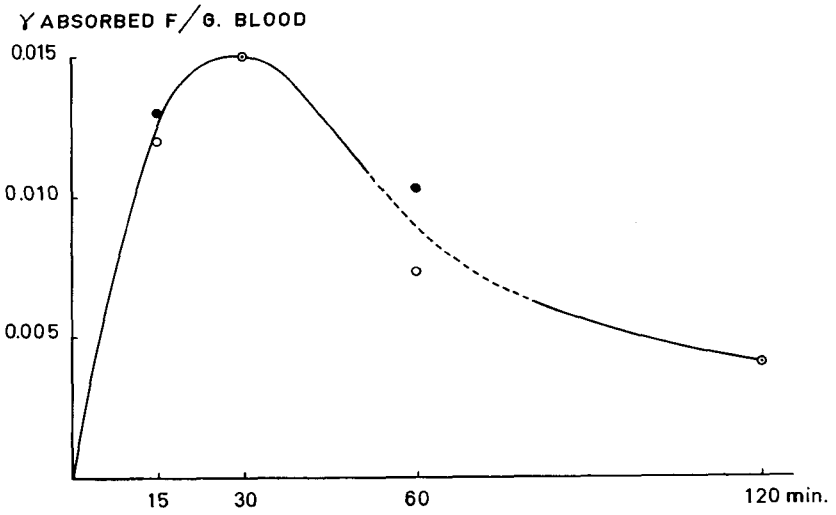

Fig. 4. Blood concentrations of absorbed fluoride in 150 g. rats.
o rats with low previous fluoride exposure
● rats with high previous fluoride exposure
⊙ coinciding points o and ●.

2. Rats of different age and weight, similar diet, peroral ingestion of dose in approximate proportion to weight.

The results of this test appear from Fig. 2. The initially higher blood content of labelled fluoride in the young rats, which appears from the diagram, was on the borderline of statistical significance while the small average difference after 30 min was non-significant. The blood of the older rats had a significantly higher fluoride content after 60 and 120 mins.

3. Rats of different age and weight, similar diet, i.v. injection of dose in approximate proportion to weight.

The results appear from Fig. 3. The blood fluoride clearance was evidently more rapid in the younger rats, with increasing, and increasingly significant, differences in percentage retention with time.

4. Rats of similar age and weight but reared on widely different water fluoride content, peroral ingestion of similar dose.

The results of the radioactive blood and bone analyses appear from Figs. 4—7. There were no clear differences between fluoride and control rats as regards either of these parameters of fluoride distribution, even though the differences of the 60 min values in Figs. 4 and 5 had P values between 0.02 and 0.05. Nor was any significant difference found at any of the time intervals as regards the unabsorbed intestinal fluoride.

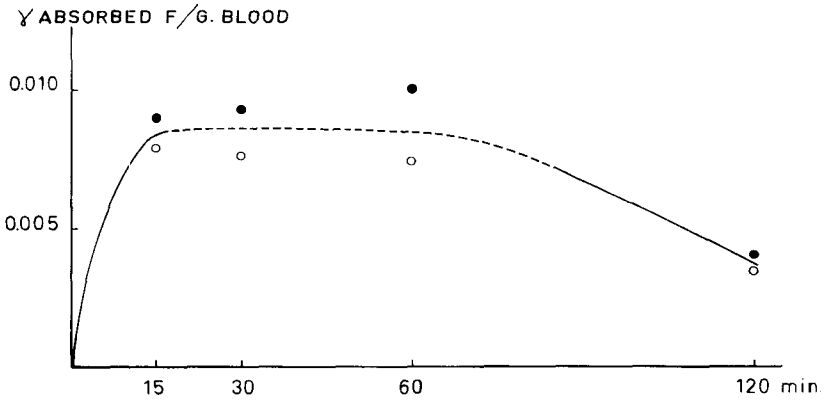

Fig. 5. Blood concentrations of absorbed fluoride in 300 g. rats. Denotations as Fig. 4.

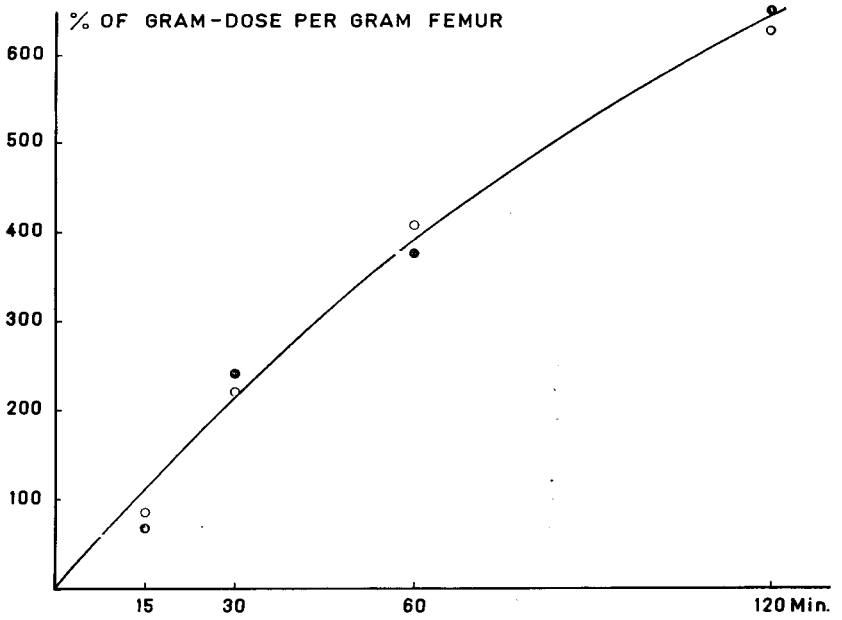

Fig. 6. Femur concentrations of labelled fluoride in 150 g. rats. Denotations as Fig. 4.

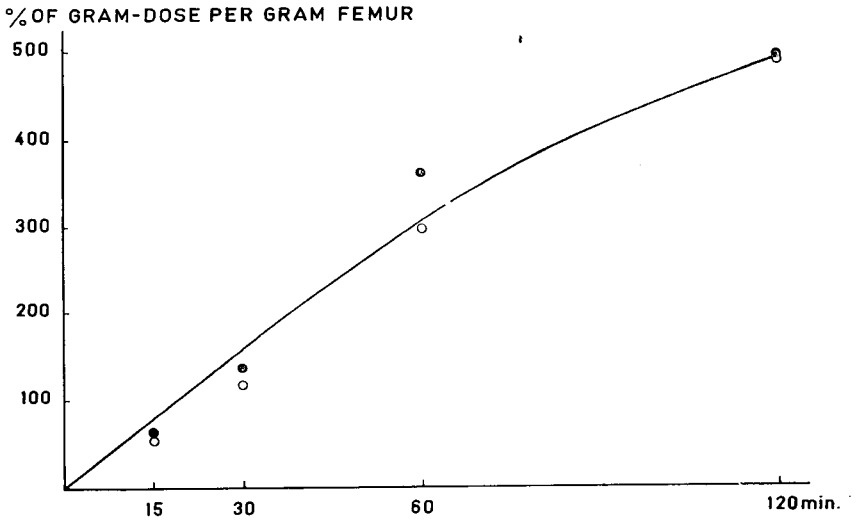

Fig. 7. Femur concentrations of labelled fluoride in 300 g. rats. Denotations as Fig. 4.

The femurs of the two younger groups of rats were found to contain 1406 ± 26.9 and 219 ± 11.6 ppm F, respectively, those of the older rats 1828 ± 82.5 and 382 ± 17.9 ppm F, respectively. The femoral uptake of labelled fluoride was, according to Figs. 6 and 7, about 25 % higher in the younger rats. Unabsorbed intestinal contents were higher at all the time intervals in the older rats, being after 120 mins. on an average 41.7 ± 4.20 % and 22.7 ± 3.07 % of the dose, respectively.

DISCUSSION

Rats of the same age and the same breeding with the exception of widely different fluoride exposure showed no clear differences as regards absorption, blood levels and femoral uptake of F^{18}-labelled fluoride. This is apparently at variance with the results of Stookey, Crane & Muhler (loc. cit.) as regards intestinal absorption. However, the experimental conditions were different: while their rats obtained 1 mg F as NaF in water solution after 24 hours' starvation, our animals were given about 8.4 μg F in 7 ml milk after about 20 hours' starvation, with access to drinking water; the latter conditions would appear to deviate less from a normal fluoride supply.

Our finding that previous fluoride exposure did not affect the femoral uptake of labelled fluoride may appear to be at variance with the data of *Suttie & Phillips* (1959) who found that the femoral uptake rate greatly decreased already after some days' fluoride ingestion. However, their rats were given about 500 ppm F in the solid food, which was "nonlethal but growth-retarding". A combination of retarded growth and skeletal fluoride saturation seems to have occurred in their rats in contrast to ours.

When old and young animals are compared, higher peak values for absorbed fluoride in the blood are regularly found in the young animals while the corresponding blood curves of the old animals are flatter. The 80 g animals given 7 ml thus had about 0.025 γ absorbed F/g blood after 15 mins. (Fig. 1), the 75 g animals given 2 ml had 0.014 γ/g after 15 mins. (Fig. 2), the 150 g animals given 7 ml had 0.015 γ/g after 30 mins.; while all the 300—330 g rats given 7—8 ml had the corresponding figures 0.007—0.01 γ/g after 15—60 mins. (Figs. 1, 2, 4 and 5).

These figures may be compared with the total blood fluoride content of the rat of about 0.10—0.15 γ/g (*Brzezinski et al.*, 1961). It must be borne in mind, however, that the figures for absorbed fluoride do not represent any net increase since the absorbed, labelled fluoride must to some extent have replaced blood fluoride that has been excreted or sequestered by the mineralized tissues.

The experimental data thus suggest the following turnover picture under the applied conditions. The rate of disappearance from the blood of absorbed fluoride is greater in younger animals, which may in part depend on the greater skeletal uptake of fluoride in the younger rats. The higher initial blood peaks of absorbed fluoride in the younger animals should depend on a more rapid intestinal absorption. The skeletal fluoride saturation that can be attained with the applied doses has no decisive influence on the absorption and blood turnover of fluoride.

SUMMARY

Rats differing in age and/or previous fluoride exposure were compared as regards the absorption, blood levels and femoral uptake of ingested F^{18}-labelled fluoride, and in one test as regards their blood clearance of intravenously injected labelled fluoride. The fluoride doses were, in most of the tests, in approximate proportion to the weight of the animal.

I.v. injected fluoride disappeared more rapidly from the blood of young rats over a 20-min period.

In spite of this, young animals showed higher maximal blood levels of absorbed fluoride.

Animals of equal age, reared on the same low-fluoride diet but with either distilled water or water containing 50 ppm F as NaF, showed no clear differences over a 2-hour period as regards absorption, blood levels or femoral uptake of ingested fluoride. However, when 82 day- and 50 day-old rats were compared, fluoride absorption was slower, blood levels lower and femoral uptake lower in the older rats.

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RÉSUMÉ

LA CLEARANCE SANGUINE DU FLUORURE CHEZ DES RATS PRÉSENTANT DES DIFFÉRENCES QUANT À L'ÂGE OU À L'EXPOSITION PRÉALABLE AU FLUORURE.

ÉTUDES UTILISANT LE FLUOR RADIOACTIF.

Chez des rats présentant des différences quant à l'âge et/ou à l'exposition préalable au fluorure on a étudié l'absorption, les taux sanguins et la concentration fémorale du fluorure ingéré, marqué de F^{18}. Dans une des expériences, la cléarance sanguine de fluorure marqué a été déterminée après injection intraveineuse. Les doses de fluorure étaient, dans la plupart des expériences, en proportion approximative au poids des animaux.

Pendant une période de 20 minutes, après injection intraveineuse, le fluorure marqué a été éliminé plus rapidement du sang des rats jeunes.

Néanmoins, chez les animaux jeunes, le taux sanguin du fluorure marqué absorbé présentait des sommets plus élevés.

Des animaux du même âge, recevant la même nourriture à faible taux de fluorure mais avec soit de l'eau distillée, soit de l'eau contenant 50 ppm de fluor sous forme de NaF, ne montrèrent aucune différence d'absorption, de taux sanguins ou de concentration fémorale de fluorure marqué pendant 2 heures après l'ingestion. Par contre, l'absorption était plus lente, les taux sanguins plus bas et les concentrations fémorales plus basses à l'âge de 82 jours qu'à l'âge de 50 jours.

ZUSAMMENFASSUNG

BLUTCLEARANCE VON RESORBIERTEM FLUOR BEI RATTEN VON VERSCHIEDENEM ALTER ODER VERSCHIEDENER FRÜHERER FLUOREXPOSITION

Ratten von verschiedenem Alter und/oder verschiedener früherer Fluorexposition wurden betreffs der folgenden Funktionen verglichen: Resorption, Blutgehalt und Femuraufnahme von peroral zugeführtem, F^{18}-markiertem Fluorid, in einem Versuch

auch betreffs der Blutclearance von intravenös eingespritztem Fluorid. Die Fluoriddosen waren in den meisten Versuchen dem Körpergewicht der Tiere etwa proportional.

Intravenös injiziertes Fluorid wurde während 20 Minuten schneller vom Blut der jungen Ratten entfernt.

Trotzdem zeigten junge Tiere höhere Maximalwerte des vom Darm resorbierten Fluorides im Blut.

Tiere von demselben Alter, die mit derselben Kost aber mit entweder destilliertem Wasser oder 50 ppm F als NaF enthaltendem Wasser aufgezogen waren, zeigten während 2 Stunden nach peroraler Fluoridzufuhr keine deutliche Unterschiede betreffs Resorption, Blutspiegel oder Femuraufnahme. Von diesen Tieren hatten aber 82 Tage alte Ratten eine langsamere Resorption, niedrigere Blutspiegel und weniger Femuraufnahme des markierten Fluors als 50 Tage alte Ratten.

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Address: *Department of Cariology,*
School of Dentistry,
Karolinska Institutet,
Stockholm, Sweden.