Effect of Molybdenum on Fluoride Retention in the Rat.* (27174)

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Adler(1) has shown that the presence of 0.1 mg of molybdenum (as ammonium molybdate) per milliliter in the drinking water reduces the incidence of dental caries in the rat, and independently, we have shown that the skeletal retention of fluoride was increased significantly when molybdenum was added to the drinking water(2). In an attempt to investigate the mechanism by which this increased skeletal retention of fluoride in the presence of molybdenum occurs, the effect of various levels of molybdenum upon the metabolism and retention of fluoride in skeleton was studied, along with an evaluation of the deposition of fluoride in the various soft tissues of the rat.

Experimental. Seventy-one weanling Sprague-Dawley strain albino rats were divided by sex and weight into 7 experimental groups of 9 animals each and a control group of 8 animals. All were housed in raised screen cages in an air-conditioned room and recevied a special, low-fluoride (F = 0.08 μg/g) stock corn diet† and re-distilled fluorine-free water *ad libitum*. Animals in Group A received no supplement. Groups B, C, and D received 0.25, 0.50 and 1.0 mg of molybdenum, respectively, as ammonium molybdate daily by stomach tube. Group E received 0.50 mg fluoride, as sodium fluoride, in an identical manner. The animals in Groups F, G, and H received, in addition to 0.50 mg fluoride, 0.25, 0.50 and 1.0 mg molybdenum, respectively. Urine and feces samples were collected on 6 animals in each group for two 48-hour periods during both the initial and the final 4 days of the 100-day experimental period. The animals were sacrificed by ether inhalation and their skins, both femurs, and the various soft tissues removed. The remaining carcasses and the femurs were individually ashed in silica dishes for 6 hours at 600°C. The soft tissues were placed individually in silica dishes and vacuum desiccated to a constant weight, after which 75 mg of a fluoride-free magnesium oxide was added to each sample. This was slurried with a small quantity of redistilled fluorine-free water, and the samples were evaporated to dryness under infra-red lamps and ashed for six hours at 600°C. All fluoride determinations were performed by means of the perchloric acid distillation method previously described(3).

Special precautions were taken during analysis of the soft tissues to eliminate fluoride contamination. These included the use of silica dishes for soft tissues only, special cleaning procedures employing boiling each with 10% HCl, followed by a thorough washing in a H_2SO_4-dichromate cleaning solution, and finally in 10% NaOH. The use of repurified MgO, special units of infra-red lamps, a muffle for soft tissue use only, and distillation and titration units which were never used for any other type of samples were added precautions. The use of these technics afforded extremely good reliability indicated by a recovery of 96.4% of added known amounts of fluoride to soft tissue samples and a reproducibility of ± 4.13%.

Results. The data obtained during the initial metabolism period for feces and urinary

TABLE I. Fluoride Content of Feces and Urine during Initial and Final Metabolism Periods.

	Initial metabolism period		Final metabolism period	
	Feces	Urine	Feces	Urine
Group	μg F/day		μg F/day	
A	2.27	9.7	8.80	7.89
B	2.10	5.0	7.83	8.42
C	3.06	10.0	7.54	6.68
D	2.30	11.2	4.61	6.63
E	10.8	118	62.1	291
F	11.6	119	53.4	297
G	6.2	139	57.2	259
H	7.6	121	35.9	287

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† Percentage composition of this diet was as follows: yellow corn meal, 64.0; powdered whole milk, 30.0; alfalfa meal, 4.8; irradiated yeast, 0.2; and iodized salt, 1.0.

TABLE II. Retention of Fluoride in Carcass and Femur of the Rat Receiving Varying Levels of Molybdenum.

Group	Carcass			Femur			Avg net % ret.†
	Total F (mg)	"t"	Conc. (ppm)	Total F (mg)	"t"	Conc. (ppm)	
A	.484 ± .057*	—	60.0 ± 4.2	.039 ± .002	—	63.0 ± 3.1	—
B	.750 ± .024	7.19	97.1 ± 5.6	.071 ± .001	23.4	116.6 ± 9.4	—
C	.610 ± .063	3.41	70.1 ± 4.4	.059 ± .005	14.6	83.9 ± 11.3	—
D	.481 ± .022	.10	59.3 ± 4.8	.054 ± .007	11.0	86.7 ± 4.1	—
E	25.5 ± .9	—	3140 ±670	2.43 ± .17	—	3970 ±360	54.8
F	27.3 ± .4	3.12	3550 ±320	2.79 ± .13	2.70	4690 ±350	58.6
G	27.4 ± .3	3.29	2910 ±540	2.65 ± .08	1.65	3590 ±230	58.8
H	26.7 ± .4	2.08	3260 ±430	2.74 ± .12	2.32	4140 ±210	57.8

* Stand. dev. of mean.
† Based upon total mg F in carcass and femur after deducting respective control values.

fluoride are found in Table I. During the initial period the fecal fluoride values for controls and those animals receiving only supplemental molybdenum were all similar. When 0.5 mg fluoride was fed, fecal fluoride increased from approximately 2.2 μg to 10.8 μg per day. When 0.5 or 1.0 mg molybdenum was fed in addition to the 0.5 mg fluoride (Groups G and H) less fluoride was found in the feces suggesting a greater fluoride absorption. This latter suggestion is supported by the fact that urinary fluoride values are similarly higher in the same animals in respect to the Group E animals which received only fluoride. Similar findings occur in older animals receiving 1.0 molybdenum and 0.5 mg fluoride (Group H) for fecal fluoride, but not for urinary fluoride as seen in the final metabolism period. The reason for these differences is undoubtedly based upon the fact that by the time the animals reached the age for the final metabolism period sufficient fluoride was ingested that its high excretory rate masked the exchange differences observed in younger animals.

The amounts of fluoride in the carcass and femur are shown in Table II. Carcasses of the control animals which received 0.25 mg molybdenum per day (Group B) contained 750 μg of fluoride, an increase of 55% compared to those animals which received no molybdenum. Carcasses of the animals which received 0.50 mg molybdenum daily (Group C) contained 610 μg of fluoride, 21% more than control animals.

Group E, which received fluoride but no molybdenum, contained 25.5 mg of fluoride, indicating a net retention of 54.8%. Group F, which received 0.25 mg molybdenum in addition to the fluoride, retained 27.3 mg of fluoride, 58.6% of amount ingested. Increasing the level of molybdenum did not result in a further increase in fluoride retention.

The femur data similarly illustrate the trends seen in the whole carcass data. The femurs of Groups B, C, and D contained significantly ($P = .01$) more fluoride than those of Group A even though none of these groups received supplemental fluoride. In those groups receiving fluoride, addition of molybdenum was associated with an increased retention of fluoride.

The analysis of the various soft tissues for fluoride is shown in Table III. These data are reported in terms of μg F/g based upon dry weight of the tissue. The soft tissues of Groups A through D, which received no supplemental fluoride, contained almost similar amounts of fluoride with the single exception of the heart. In the hearts of the control ani-

TABLE III. Soft Tissue Data (Average μg F/g Dry Wt).*

Group	Muscle	Liver	Heart	Kidney	Brain
A	1.85	.80	1.17	1.73	3.87
B	1.50	.83	2.64	1.82	3.06
C	1.68	1.08	2.26	1.84	3.24
D	1.79	.82	2.69	1.61	2.93
E	1.61	1.41	3.19	2.47	3.82
F	2.90	1.63	3.53	3.52	6.31
G	2.88	1.54	3.39	3.83	5.85
H	2.80	1.65	3.59	3.75	15.18

* Each value represents mean value obtained by pooling 3 samples of each tissue for analysis. This was necessary since such microquantities of fluorine are found in the soft tissues that accurate individual organ analysis is not highly reliable. Such a procedure eliminates the possibility of calculating a value for stand. dev. (see *J. Nutrition*, 1958, v65, 265).

mals a level of 1.17 ppm was found while those from Groups B, C, and D animals which received supplemental molybdenum were found to contain 2.64, 2.26 and 2.69 ppm fluoride. Comparison of the values obtained in Group E with those in Group A indicates that in liver, heart, and kidney, administration of 0.5 mg of fluoride resulted in increased fluoride content of 0.61, 2.02, and 0.74 μg/g, respectively. No differences were found in fluoride content of the brain when 0.5 mg was administered, and less fluoride was found in muscle under similar conditions. Addition of molybdenum resulted in a further increase in concentration of fluoride in all of the soft tissues as compared with values obtained in Groups F, G, and H with those of Group E. This effect was least apparent in liver and heart and most pronounced in muscle and brain.

Discussion. The data obtained during the initial metabolism period support earlier studies in our laboratory which suggested that the presence of molybdenum in young albino rats is not associated with an increased retention of fluoride in the carcass. However, the data obtained in this study in older animals during the final metabolism period suggest that while 0.25 mg of molybdenum (Group F) did not appear to increase fluoride retention when compared to animals receiving no molybdenum but 0.5 mg fluoride, levels of 0.5 and 1.0 mg of molybdenum did result in an increased retention of fluoride. These data do not indicate the mechanism of this increased retention. In animals receiving the higher levels of molybdenum the increased fluoride retention may be a result of a decreased urinary fluoride excretion or an increased rate of absorption indicated by a decreased fecal fluoride content. The data obtained from the whole carcass and femur analysis support the metabolism data and indicate an increased retention of fluoride in the presence of molybdenum.

The effect of molybdenum is not limited to the skeleton; fluoride deposition is also increased in soft tissues. When no supplemental fluoride was administered, the presence of molybdenum did not appreciably alter the deposition of fluoride except in the heart. In this organ the presence of increasing levels of molybdenum from 0.25 to 1.0 mg resulted in 125.8, 93.0, and 129.9% increases in concentration of fluoride in the dry tissue. When supplemental fluoride was administered, liver, heart, and kidney showed increased fluoride concentrations of 76.2, 172.8, and 42.8%. Administration of increasing levels of molybdenum did not appreciably alter the concentration of fluoride in liver and heart although both tissues indicated a trend towards an increased deposition in presence of molybdenum. In the muscle, however, increasing levels of molybdenum from 0.25 to 1.0 mg were accompanied by 80.2, 78.9, and 73.9% increases in concentration of fluoride when compared to those animals which received only the supplemental fluoride. A similar comparison in the kidney shows increased fluoride concentrations of 42.5, 55.1, and 51.8 associated with the increasing levels of molybdenum. The most pronounced influence of molybdenum upon the soft tissue deposition of fluoride may be seen in the brain. Here the increasing levels of molybdenum from 0.25 to 1.0 mg are associated with 65.2, 53.2, and 298% increases in fluoride concentrations of the dry tissue.

These data suggest that in the absence of supplemental fluoride, molybdenum does not appreciably alter the deposition of fluoride except in the heart. In the presence of elevated levels of fluoride, the presence of molybdenum is associated with an increased concentration of fluoride in muscle, kidney, and brain, although the increases in deposition of fluoride do not appear to be proportional to amount of molybdenum administered.

A comparison of the data obtained during the initial and final metabolism periods provides information as to skeletal retention at different ages. It is important in human nutrition to know whether a greater proportion of dietary fluoride is retained by children than by adults. It is the consensus of opinion, although adequate proof is lacking, that 95% of the ingested fluoride is excreted(4). While findings obtained from animal data cannot be translated to human nutrition, the data obtained in this study as well as in others(5) clearly suggest that not only is sig-

nificantly more than 5% of ingested fluoride retained by the body, but that retention is a function of age and previous exposure to the element. During the initial metabolism period the animals in this study were 24-28 days of age and were in a rapid stage of skeletal growth. The fluoride data obtained at this time (Table I) indicate that about 75% of the ingested fluoride was incorporated into the skeleton. During the final metabolism period the animals were 120-124 days of age and were in an early stage of maturation which is accompanied by a significantly decreased rate of growth and skeletal exchange. The fluoride data obtained at this time indicate that only 30% of the ingested fluoride was deposited in the skeleton. These data thus suggest not only a decreased rate of fluoride retention as a function of increased age, but in addition, a possible mechanism for the action of molybdenum upon fluoride metabolism.

Summary. The results suggest that administration of molybdenum increased retention of fluoride, but that this effect is more prominent in older animals. Analysis of the soft tissues showed that molybdenum significantly increased concentration of fluoride. However, elevations in fluoride content were not directly proportional to increases in amount of molybdenum administered.

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Activation of *Bartonella muris* Infection in X-Irradiated Rats. (27175)

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Bartonellosis, caused by *Bartonella muris*, is an endemic disease in many rat colonies. The infected animals are essentially free of symptoms unless splenectomized. It has been reported(1,2) that latent *Bartonella muris* infection in the rat may be activated by exposure to whole body X-irradiation. The presence of this disease may be a complicating factor in radiation experiments when studying diverse protective procedures that may modify the response to the *Bartonella muris* infection. In this investigation, the effect of X-irradiation on latent bartonellosis in the rat was confirmed and the infectivity of whole blood, red blood cells and plasma containing this organism from infected X-irradiated rats was compared.

Methods. Infected donor animals. Female albino rats weighing 80-100 g were obtained from the Royal Hart breeders, Middletown, N. Y. The Royal Hart rats had *Bartonella muris* infection as diagnosed following splenectomy by anemia, presence of Bartonella bodies in the blood smear and death of the animal. The donor animals were exposed to 2 doses of whole body radiation at 3-day intervals. Each dose was equivalent to 250 rad. Ten to 15 rats were placed in a flat plastic cylindrical container so that movement was limited to a minimum and only in a horizontal plane during the exposure period. X-radiation was delivered by 250 KVP X-ray unit operating at 30 milliamperes with 1.0 mm Cu and 1.0 mm Al filters. Using an absorption value of 0.58 as an average quantity over the plastic container and a transmission factor of 94% for the ⅛″ thick plastic top, as determined by dosimetry measurements, average dose rate was 51 rad per minute.

Uninfected receptor animals. Female CFN rats weighing 80-100 g were obtained from