Effect of Molybdenum on Fluoride Absorption.* (27275)

GEORGE K. STOOKEY, DANIEL B. CRANE AND JOSEPH C. MUHLER
Department of Biochemistry, Indiana University Medical Center, Indianapolis

Adler has shown that the presence of molybdenum in drinking water is associated with a reduced incidence of dental caries in rats(1). Previous studies in our laboratories have shown that addition of 50 ppm molybdenum to the drinking water of rats is associated with significantly more fluoride retention both in the skeleton and whole carcass than occurs in the absence of molybdenum (2). To learn more concerning the mechanism by which this increased fluoride retention occurs, studies were designed to determine the effects of molybdenum on gastrointestinal absorption of fluoride.

Methods. Series I. A total of 139 Sprague-Dawley strain albino rats, ranging in age from 40 to 275 days, bred and raised in our laboratories, were divided into 3 groups. Group A received fluoride, Group B, fluoride plus molybdenum, and Group C served as a control. Each group was divided according to age and body weight into subgroups representing different ages for studying absorption of fluoride from the stomach (Table I), the first 5 cm of the duodenum (Table II), and the more distal portions of the intestine (Table III). All those in Group A received a specified amount of fluoride ranging from 50 μg to 1.0 mg. The animals in Group B received the same amount of fluoride, plus molybdenum ranging from 125 μg to 1.0 mg. All animals in Group C received no fluoride or molybdenum.

All of the animals were housed in raised screen wire cages in an air-conditioned room and received a special low-fluoride diet (F = 0.5 ppm) and redistilled fluoride-free water *ad libitum*. Prior to surgery all animals were starved 24 hours. Each animal was anesthetized with sodium pentobarbital and the abdomen surgically opened. The segment of the gastrointestinal tract to be studied was carefully and completely ligated superiorly and inferiorly with a minimal amount of vascular disturbance. The isolated segment was injected with either a solution of fluoride (as NaF) or a solution containing both fluoride and ammonium molybdate. The injected segment was allowed to remain in the intact anesthetized animal for exactly 30 minutes in all instances. The entire segment was then removed and placed in a fused silica crucible containing 0.30 g of a low-fluoride (F = 0.48 μg/g) calcium oxide. A small quantity of redistilled fluoride-free water was added to the crucible in order to slurry the contents in the calcium oxide solution. The crucible and its contents were then evaporated to dryness under infra-red lamps and ashed in a preheated muffle at 600°C for 6 hours. The ashed samples were quantitatively transferred to a distilling flask and the fluoride determined by perchloric acid distillation and titration of the distillate with acidified thorium nitrate using alizarin as an indicator(3).

Series II. Since the data obtained in Series I suggested that the age of the animal is a factor in fluoride absorption, and since anesthesia is known to slow metabolic processes, it was decided to repeat a portion of the studies employing different experimental technics. In Series II, adult albino rats 280 to 320 days of age were starved for 36 hours. One-half of the animals were given 1.0 mg of fluoride (as NaF) in 1.0 ml of water by stomach tube and the remaining animals received in an identical manner 1.0 mg molybdenum as ammonium molybdate in addition to the fluoride. All of the animals remained under surgical anesthesia during the post stomach tube period. After 2 hours the animals were sacrificed and the gastrointestinal tract removed and analyzed for fluoride as previously described (Table IV). Similarly, adult albino rats, 200 to 320 days of age were divided into 2 groups as above, but instead of remaining under surgical anesthesia following administration of either fluoride or molybdenum, the animals were not anesthetized until the 30-minute study period passed

* This study was supported in part by a grant from U. S. Public Health Service.

TABLE I. Fluoride Absorption from the Stomach (Series I).

Group		No. of animals	Mean final wt (g)	μg F inj.	μg Mo inj.	Total μg F found	Net μg F found	Avg % absorption	t	p	Effect of Mo (%)
I	A*	5	352	200	—	156.0	141.7 ± 6.1†	29.7	—	—	—
	B	5	347	200	500	162.5	148.2 ± 4.3	25.9	1.80‡	.1	−12.8
	C	3	361	—	—	14.3	—	—	—	—	—
II	A	6	193	400	—	269.8	254.8 ± 8.3	36.3	—	—	—
	B	6	188	400	1000	296.7	281.7 ±10.1	29.5	2.67	.02	−18.7
	C	3	180	—	—	15.0	—	—	—	—	—
III	A	6	120	100	—	73.7	64.7 ± 4.1	35.3	—	—	—
	B	6	130	100	250	69.7	60.7 ± 5.3	39.3	1.02	.3	+11.3
	C	3	127	—	—	9.0	—	—	—	—	—
IV	A	3	72	100	—	78.4	74.4 ± 4.6	25.6	—	—	—
	B	3	83	100	250	78.5	74.5 ± 3.9	25.5	.04	1.0	None
	C	2	80	—	—	4.0	—	—	—	—	—

* A = F; B = Mo + F; C = Control. † Stand. dev. ‡ Calculated from net μg F.

TABLE II. Fluoride Absorption from the Intestine (Series I, Using First 5 cm Segment).

Group		No. of animals	Mean final wt (g)	μg F inj.	μg Mo inj.	Total μg F found	Net μg F found	Avg % absorption	t	p	Effect of Mo (%)
I	A*	3	382	100	—	86.8	80.5 ± 2.9†	19.5	—	—	—
	B	3	408	100	250	77.8	71.5 ± 3.7	28.5	2.38	.05	+46.2
	C	2	380	—	—	6.3	—	—	—	—	—
II	A	3	357	100	—	76.0	69.3 ± 3.4	30.7	—	—	—
	B	3	342	100	250	71.9	65.2 ± 2.8	34.8	.91	.4	+13.4
	C	2	349	—	—	6.7	—	—	—	—	—
III	A	3	120	100	—	57.6	50.7 ± 4.8	49.3	—	—	—
	B	3	130	100	250	69.7	62.8 ± 4.3	37.2	2.41	.05	−24.6
	C	2	127	—	—	6.9	—	—	—	—	—
IV	A	3	83	50	—	32.7	28.4 ± 2.9	43.2	—	—	—
	B	3	72	50	125	35.7	31.4 ± 3.4	37.2	.69	.5	−13.9
	C	2	78	—	—	4.3	—	—	—	—	—

* A = F; B = Mo + F; C = Control. † Stand. dev.

TABLE III. Fluoride Absorption from Intestinal Segments (Series I, Using Second and Third 5 cm Segment).

Group		No. of animals	Mean final wt (g)	μg F inj.	μg Mo inj.	Total μg F found	Net μg F found	Avg % absorption	t	p	Effect of Mo (%)
† I	A*	3	384	100	—	62.6	50.8 ± 3.9§	49.2	—	—	—
	B	3	408	100	250	57.7	45.9 ± 4.2	54.1	1.19	.3	+ 9.7
	C	2	386	—	—	11.8	—	—	—	—	—
† II	A	3	79	100	—	64.9	59.8 ± 5.1	40.2	—	—	—
	B	3	72	100	250	47.8	42.7 ± 3.8	57.3	2.47	.05	+43.5
	C	2	75	—	—	5.1	—	—	—	—	—
‡ III	A	3	373	100	—	45.0	35.7 ± 4.2	35.7	—	—	—
	B	3	347	100	250	56.4	47.1 ± 4.0	47.1	1.79	.1	+32.0
	C	2	358	—	—	9.3	—	—	—	—	—
‡ IV	A	3	145	100	—	57.9	51.7 ± 4.1	48.3	—	—	—
	B	3	152	100	250	51.7	45.5 ± 3.0	54.5	1.21	.2	+12.8
	C	2	144	—	—	6.2	—	—	—	—	—

* A = F; B = Mo + F; C = Control.
† Groups I & II—2nd 5 cm segment studied. ‡ Groups III & IV—3rd 5 cm segment studied.
§ Stand. dev.

TABLE IV. Fluoride Absorption from Entire G-I Tract When Animals Remained Anesthetized during 2-Hour Absorption Period (Series II).

Group	No. of animals	Mean final wt (g)	μg F inj.	μg Mo inj.	Total μg F found	Net μg F found	Avg % absorption	t	p	Effect of Mo (%)
A*	6	441	1000	—	569.9	548.2 ± 30.7†	45.2	—	—	—
B	6	423	1000	1000	449.7	428 ± 24.9	57.2	3.87	.01	+26.6
C	3	460	—	—	21.7	—	—	—	—	—

* A = F; B = Mo + F; C = Control. † Stand. dev.

TABLE V. Fluoride Absorption from Entire G-I Tract without Anesthesia during 30-Minute Absorption Period (Series II).

Group	No. of animals	Mean final wt (g)	μg F inj.	μg Mo inj.	Total μg F found	Net μg F found	Avg % absorption	t	p	Effect of Mo (%)
A*	6	424	1000	—	728.9	701.0 ± 23.4†	29.9	—	—	—
B	6	431	1000	1000	629.7	601.8 ± 31.0	39.8	3.31	.01	+33.1
C	3	407	—	—	27.9	—	—	—	—	—

* A = F; B = Mo + F; C = Control. † Stand. dev.

(Table V). During the absorption period all of the animals were housed individually without food or water.

Results. The results are shown in Tables I-V. Table I shows that the presence of molybdenum is seen to decrease numerically the absorption of fluoride from the stomach in adult animals (approximately 200 days of age) by 12.8% and in younger animals (approximately 75 days of age) by 18.7%. In still younger animals (ranging in age from 40 to 45 days) the presence of molybdenum is found either to increase absorption of fluoride (Group III) or to have no appreciable effect (Group IV). Since the concentration of both fluoride and molybdenum administered was not constant, the effect of fluoride absorption from the stomach as a function of age of the animal in presence and absence of molybdenum cannot be determined exactly from these data.

The data in Table II concern absorption of fluoride from the 5 cm segment of the intestine immediately distal to the stomach (essentially the duodenum). The presence of molybdenum increased the absorption of fluoride in adult rats (about 200 days of age) by 46 and 13% ($p = 0.05$ and 0.4, respectively). However, in younger animals (approximately 75 and 40 days of age) the absorption is decreased by 25 and 14% ($p = 0.05$ and 0.5, respectively). In segments further removed from the stomach (Table III) the presence of molybdenum increased fluoride absorption in all cases irregardless of age of the animal.

The results obtained in Series II are shown in Tables IV and V. The data in Table IV indicate that molybdenum increases the rate of fluoride absorption throughout the entire gastrointestinal tract to what would be expected from the summation of effects produced in this age group from the combined data obtained in Series I. The data in Table V suggest that anesthesia has little, if any, effect upon rate of fluoride absorption since the value obtained in this portion of the study was comparable to that obtained on animals of this age in Series I under similar conditions when anesthesia was employed.

Discussion. These results suggest that one possible explanation for the increased retention of fluoride in the skeleton in presence of molybdenum may be due to the ability of molybdenum to increase the metabolic availability of fluoride by increased absorption in the intestine, although this occurs predominantly in adult animals. In adult animals (approximately 200 days of age) the presence of molybdenum is associated with a 12.8% decrease in absorption of fluoride from the stomach, but with an increase in fluoride absorption from the intestine ranging from 13.4 to 46.2%.

In younger animals (approximately 75 days of age) the presence of molybdenum is associated with a 11% decrease in rate of fluoride absorption from the stomach, a 25% decrease in the first 5 cm segment of the intestine, but a 13% increase in the third 5 cm segment. Thus, in animals approximately 75 days of age, the segments studied indicate that the presence of molybdenum is associated with a net decrease of 23% in rate of fluoride absorption.

No consistent finding was noted by altering the amount of molybdenum from 125 μg to 1.0 mg. This corroborates our previous conclusions that concentrations of molybdenum ranging from 250 to 1000 μg per day given to rats provided comparable increases in retention of fluoride(2). In this latter study the influence of molybdenum upon fluoride metabolism was studied in weanling and adult rats by an evaluation of fluoride in the urine and feces. The data indicated that in weanling animals the presence of molybdenum did not appreciably increase absorption, but in older animals (124-128 days of age) the presence of molybdenum was associated with an increased absorption of fluoride.

When the entire gastrointestinal tract (Table IV) is employed to evaluate fluoride absorption, the presence of molybdenum is associated with an overall increase of 27% in amount of fluoride absorbed in adult rats after 2 hours. These data confirm the findings obtained by using intestinal segments and further indicate that the effect of molybdenum is prolonged over at least a 2-hour period following ingestion. The data shown in Table V indicate that when the animals were not maintained under ether anesthesia during the absorption period (as were all of the animals in series 1) the presence of molybdenum was associated with a 33% increase in absorption of fluoride from the entire gastrointestinal tract of adult albino rats. These data suggest that ether anesthesia has no appreciable effect on either fluoride absorption, nor upon the effect of molybdenum upon fluoride absorption, since the rate of fluoride absorption shown is comparable to that found in the stomach and various segments of the duodenum in presence of molybdenum.

Summary. Studies were conducted to investigate the mechanism by which molybdenum increases the skeletal retention of fluoride. The absorption of fluoride in presence and absence of molybdenum was studied in albino rats ranging in age from weanling to adults, employing isolated gastrointestinal tract segments and the entire tract in the intact animals. These data indicate that age plays a prominent role in absorption of fluoride as affected by molybdenum. In general, the presence of molybdenum significantly ($p = 0.05$) increases the absorption of fluoride from the first portion of the intestine of older animals and acts inversely in young animals. The reverse is seen in the stomach where molybdenum appears to decrease absorption of fluoride in older animals, and either increases it slightly or has no effect in younger animals. These data suggest that in older animals the presence of molybdenum may increase absorption of fluoride.

1. Adler, P., *Odont. Revy.*, 1957, v8, 202.

2. Stookey, G. K., Muhler, J. C., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 379.

3. Weddle, D. A., Muhler, J. C., *J. Nutr.*, 1954, v54, 437.

Received August 7, 1961. P.S.E.B.M., 1962, v109.