PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **155**, 23-26 (1977)

Influence of Variations in Fluoride Intake on the Ionic and Bound Fractions of Plasma and Muscle Fluoride (39736)[1]

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The concept of "ionic" and "bound" fractions of plasma fluoride has existed for some time (1, 2). This concept originally emerged from the observation that the values determined for the total fluoride content of plasma were higher than those obtained by procedures which measure ionic fluoride. Although numerous studies have monitored changes in the total fluoride content of plasma and other tissues following variations in dietary fluoride intake (3–5), there is a rather surprising lack of information with regard to the changes in the concentration of the ionic and bound fractions which make up the total fluoride content of the specimen.

Thus a study was undertaken to monitor the changes in the ionic, bound, and total fluoride content of plasma and muscle when rats maintained on a low fluoride intake were subjected to a large increase in dietary fluoride followed, after a time, by a sharp reduction in fluoride intake.

Methods. Male Holtzman rats (113) were obtained as weanlings and provided deionized water and a low fluoride diet (6) containing 0.4 ppm of fluoride. After 14 days, 14 rats were sacrificed (Day 0 controls) and the remaining rats were transferred to drinking water containing 25 ppm of fluoride added as sodium fluoride. A total of 51 rats were sacrificed 1, 2, 4, 10, and 28 days following the dietary transfer. At the end of the 28-day period of high fluoride intake the remaining rats were transferred from the 25 ppm drinking water to deionized water and representative rats were sacrificed 1, 2, 4, 10, and 28 days later.

Blood was obtained by heart puncture and collected in heparinized tubes. The plasma was collected after centrifugation and stored frozen until analyzed. The humeri were removed and a pooled sample of muscle was obtained from several body locations on each rat. The humeri were thoroughly cleaned of all soft tissue and marrow and defatted with a 1:1 mixture of absolute ethanol and anhydrous diethyl ether in a Soxhlet apparatus for 48 hr. The fat-free humeri were ashed for 16 hr at 500°, and ground to a fine powder. Approximately 5 mg of bone ash was dissolved in 1 ml of 1.25 *N* hydrochloric acid, 0.5 ml of 0.05 *M* sodium acetate was added, and the pH was adjusted to 5.0 with 0.125 *N* sodium hydroxide. The solution was diluted to 5 ml with redistilled water and the fluoride content of the solution was determined with an Orion fluoride-ion electrode (7).

Samples of plasma were ashed at 500° with 10 mg of calcium phosphate added as a fluoride fixative. The total fluoride content in the ashed plasma was determined colorimetrically after isolation of the fluoride by diffusion from perchloric acid (8).

The ionic fluoride content of the plasma was measured by a modification of the procedure described by Singer and Armstrong (9). Plasma (1 ml) was added to 1 ml of buffer (0.05 *M* acetate, 0.11 *M* sodium chloride, pH 5.0) and the pH was adjusted to 5.0 by the addition of a small quantity of 1 *M* acetic acid. The fluoride content of this solution was measured with an Orion fluoride-ion electrode.

The ionic fluoride content of muscle was measured in an ultrafiltrate collected through Amicon CF50A membranes which retain molecules with a molecular weight greater than 50,000 after mincing 5 g of the fresh specimen, adding 2 ml of water, and homogenizing the mixture with a Thermovac homogenizer. Ultrafiltrate (1 ml) was added to 2 ml of buffer (0.05 *M* acetate, 0.11 *M* sodium chloride, pH 5.0), the pH was adjusted to 5.0 with 1 *M* acetic acid,

[1] This investigation was supported by Public Health Services Research Grant DE-01850 from the National Institute of Dental Research.

ISSN 0037-9727

and the fluoride content of the solution was measured with the fluoride-ion electrode. Appropriate corrections for the dilution of the tissue water were made.

The total fluoride content of the muscle was isolated by diffusion from a perchloric acid extract of dried, ground muscle powder (8). The diffusates (0.5 ml of 0.5 *N* sodium hydroxide) were transferred from the diffusion plate to a polyethylene beaker using several rinses of redistilled water. 0.1 ml of 1 *M* acetic acid was added, and the pH was adjusted to 5.0 by the addition of a small quantity of 2.25 *N* hydrochloric acid. The solutions were diluted to 4 ml and the fluoride contents were measured with an Orion fluoride-ion electrode.

Groups were statistically compared by calculating the Student's *t* value (10). A *P* value of less than 0.025 was chosen as indicating significance.

Results and discussion. The percentage of fluoride in the ash and the total fluoride content of the humeri for the various time points are shown in Table I. The fluoride load of the animals was indeed low prior to the period of high fluoride intake since the humeri contained a mean total of only 3.5 μg of fluoride on Day 0. During the 28-day period of high fluoride intake the fluoride load of the rats increased dramatically as evidenced by the fact that the total fluoride content of the humeri increased by over 100-fold to 415 μg. During this interval the concentration of fluoride in the humeri increased from 0.004 ± 0.0003 to 0.191 ± 0.0048% of the ash weight. After being placed on low fluoride intake for 28 days, approximately 19% of the total fluoride of the humeri was mobilized, with nearly all of the loss occurring within the first 10 days following the dietary transfer. This observation corresponds well with earlier studies indicating that part of the skeletal fluoride is rapidly turned over and excreted but that most is much more difficult to mobilize (3, 11). The apparently lower total fluoride content of the humeri of the rats sacrificed 1 day after the transfer from high to low fluoride intake is probably a reflection of the fact that these bones were slightly smaller than those from the rats sacrificed after 28 days of high fluoride intake.

Figure 1 presents the ionic, bound, and total fluoride content of the plasma at various times during the study. The ionic and total fluoride levels of the plasma were very low prior to the period of high fluoride intake, 0.005 ± 0.007 and 0.03 ± 0.005

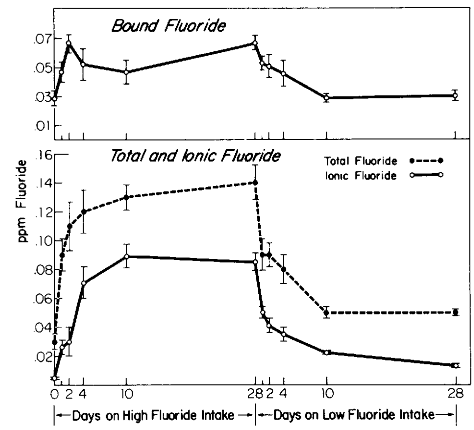

FIG. 1. Effect of variations in fluoride intake on the bound (upper panel), ionic, and total (lower panel) fluoride levels of plasma.

TABLE I. FLUORIDE CONTENT OF THE HUMERI DURING PERIODS OF HIGH AND LOW FLUORIDE INTAKE[a].

Fluoride intake	Day of study	Fluoride concentration in the humeri (% of ash weight)	Total fluoride in the humeri (μg)
Low	0	0.004 ± 0.0003 (14)	3.5 ± 0.30 (14)
High	1	0.021 ± 0.0011 (10)	17.3 ± 0.70 (10)
	2	0.035 ± 0.0014 (11)	29.8 ± 1.08 (11)
	4	0.055 ± 0.0029 (10)	49.8 ± 3.30 (10)
	10	0.123 ± 0.0018 (10)	148 ± 5.3 (9)
	28	0.191 ± 0.0048 (10)	415 ± 13.9 (10)
Low	1	0.170 ± 0.0047 (11)	359 ± 14.8 (11)
	2	0.168 ± 0.0026 (9)	373 ± 10.3 (10)
	4	0.163 ± 0.0052 (10)	378 ± 16.7 (10)
	10	0.132 ± 0.0036 (9)	343 ± 17.1 (10)
	28	0.102 ± 0.0020 (9)	338 ± 7.3 (9)

[a] Mean ± SEM for the number of animals indicated in the parentheses.

ppm, respectively. Immediately following the transfer of the rats to high fluoride intake a dramatic and rapid increase occurred in the ionic and total fluoride levels of the plasma. The ionic and total plasma fluoride levels had reequilibrated at higher levels (0.09 ± 0.008 and 0.13 ± 0.009 ppm) approximately 10 days after the dietary transfer. No significant changes in the ionic or total fluoride content of the plasma were observed over the next 18 days of high fluoride intake. Immediately following the transfer back to low fluoride intake there was a precipitous drop in the ionic and total fluoride levels of the plasma. Again, approximately 10 days were required for reequilibration. The long period of time required for reequilibration of plasma fluoride levels observed in this study is in marked contrast to that observed in humans by Singer *et al.* (12). They reported the total plasma fluoride levels of humans given 20 mg of fluoride daily in two divided doses for 114 days returned to pretreatment levels within 24 hr after the last dose. They observed that the bound fluoride levels returned to pretreatment values within 24 hr although the ionic fluoride levels showed a two- to threefold increase over the pretreatment levels. A major reason for the difference in results may be the fact that, on a body weight basis, the daily fluoride intake in the present study was undoubtedly much higher than that employed by Singer *et al.* It should be emphasized that even after 28 days of low fluoride intake, the ionic and total plasma fluoride levels (0.013 ± 0.004 and 0.05 ± 0.007 ppm, respectively) were still significantly higher than those observed prior to the period of high fluoride intake ($P < 0.0005$ for ionic fluoride and $P < 0.025$ for total fluoride). This observation is probably due to the fact that significant quantities of fluoride were still being mobilized from bone and, presumably, from other tissues.

The quantity of bound fluoride in the plasma is a calculated value obtained by subtracting the ionic fluoride from the total fluoride level. On Day 0 the quantity of bound fluoride in the plasma was 0.029 ± 0.0046 ppm. Within one day after the transfer to high fluoride intake a significant increase ($P < 0.025$) was noted in the quantity of bound fluoride in the plasma to 0.047 ± 0.0072 ppm. Significantly elevated quantities of bound fluoride were maintained throughout the entire period of high fluoride intake (P values ranged between 0.025 and 0.0005) with the maximum level (0.067 ± 0.0062 ppm) being reached within 2 days of the dietary transfer. Venkateswarlu has examined the quantity of bound fluoride in a number of pools of bovine and human serum (13). His reported values for bound fluoride, which ranged between 0.02 and 0.07 ppm, are in excellent agreement with the values found in this study. Immediately following the transfer back to low fluoride intake the quantity of bound fluoride in the plasma declined gradually with the baseline level being attained between 4 and 10 days. There was no further decrease in the quantity of bound fluoride over the last 18 days of low fluoride intake. The data clearly indicate that component(s) in plasma are capable of binding very significant quantities of fluoride following an increase in fluoride intake and of releasing this fluoride when fluoride intake is reduced. The binding component(s) are apparently saturated within two days following the transfer to high fluoride intake. The exact nature of these binding component(s) is not known although we have previously reported that bovine plasma contains fluoride bound to a component with a molecular weight of less than 1000 (14).

The data obtained for the ionic, bound, and total fluoride content of the muscle are given in Fig. 2. The results for each time point are expressed as micrograms of fluo-

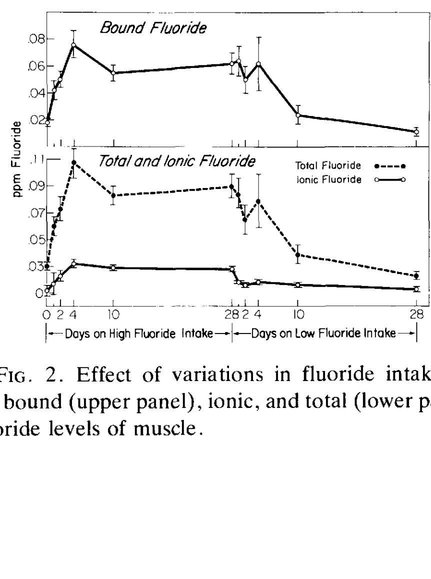

FIG. 2. Effect of variations in fluoride intake on the bound (upper panel), ionic, and total (lower panel) fluoride levels of muscle.

ride per gram of fresh muscle. The ionic and total fluoride contents of the muscle on Day 0 (0.012 ± 0.0012 and 0.030 ± 0.0023 ppm, respectively) were significantly increased to 0.028 ± 0.0024 and 0.090 ± 0.0090 ppm, respectively, by 28 days of high fluoride intake ($P < 0.0005$ for ionic and total fluoride). Although the changes in the ionic and total fluoride content of muscle were smaller than that observed in plasma, the time course of the changes was nearly identical. Approximately 10 days were required for reequilibration of the muscle ionic and total fluoride levels. Twenty-eight days following the transfer to low fluoride intake the ionic (0.013 ± 0.0018 ppm) and total (0.023 ± 0.0032) fluoride levels had returned to those observed prior to the period of high fluoride intake. Again, as was observed for plasma, 4–10 days were required for complete reequilibration.

Within 1 day following the transfer to high fluoride intake a very significant increase ($P < 0.0025$) was observed in the quantity of bound fluoride in muscle. The maximum levels of bound fluoride (0.076 ± 0.0099 ppm) were attained within 4 days following the dietary transfer. In contrast to plasma, most of the increase in the total fluoride content of muscle was due to an increase in the bound fraction rather than the ionic fraction. After 28 days of low fluoride intake the quantity of bound fluoride in muscle had decreased to the levels observed on Day 0. Reequilibration of the bound fluoride levels was complete by 10 days following the dietary transfer.

Summary. The changes occurring in the ionic, bound, and total fluoride contents of plasma and muscle from rats shifted from low fluoride intake to high fluoride intake and back to low fluoride intake were examined. Approximately 10 days were required for the reequilibration of the ionic and total fluoride levels of plasma and muscle following changes in the dietary fluoride intake. Data have been obtained that show the systematic changes produced in the bound fraction of plasma and muscle fluoride by variations in dietary fluoride intake.

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Received September 1, 1976. P.S.E.B.M. 1977, Vol. 155.