The Relation of Fluoride to Other Inorganic Substances *in vivo*.* (30471)

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The recognition of toxicity produced by high concentrations of fluoride in humans was reported as early as 1899(1). In 1932 Møller and Gudjonsson(2) first described pathological changes in the bones in workers engaged in crushing and refining cryolite and ascribed the changes to ingestion of fluoride. The curious sclerosis which appeared in the bones of the affected subjects did not cause any trouble unless the ligaments and the muscular attachments had become involved. Møller and Gudjonsson suggested the use of cryolite or of "some compound of fluorine such as sodium fluoride" to treat bone diseases that produced pronounced osteoporosis. They quote the work of Brissemoret(3) in which similar therapy for bone diseases had been proposed. Rich and Ensinck(4) reported results of observations in patients with osteoporosis or with Pagets' disease who had been treated with sodium fluoride over a period of 14 weeks. The work described here, in which rats were used as experimental animals, shows that ingestion of fluoride can produce alterations in the absorption and excretion of minerals. Differences in mineral content of bone and of teeth when a high concentration of fluoride is given have also been demonstrated.

Materials and methods. Thirty-one female rats (Charles River Laboratories) of mean weight 40 g were placed on a synthetic diet (5) with fluoride omitted from the trace mixture used. Analysis of the diet for fluorine showed the concentration to be 1.02 p.p.m. The diet was prepared and tube fed as previously described(6). Each animal initially received 4 g of the diet per day, the amount being gradually increased until the fifth day when 8 g were fed. The food intake thereafter was maintained at 8 g.

After 18 days on the diet the rats were divided into 2 groups, one of which (Group I with 16 rats) continued to receive the same diet as previously and served as control animals. The other 15 rats (Group II) had the same diet as Group I with sodium fluoride added so that the concentration of F^- was 0.001%. After 12 days and again after 20 days 12 of the animals from Group I, and 11 animals from Group II were placed in metabolic cages(6) and urine and faecal specimens were collected for 2 daily periods. The excretion of calcium and of magnesium was measured in both the urine and faecal specimens by methods previously described (7). Inorganic phosphate was measured by the method of Sumner(8). The presence of fluoride in concentrations up to 1% did not show any interference in these or subsequently described analytical methods. The rats in Group II were then given a diet containing 0.035% F^-. After 7 and again after 14 days on the diet with increased fluoride balance studies were again carried out for 2 daily periods. At the end of the last period 8 rats from each group were sacrificed and the 2 femurs removed and a blood sample collected. The remaining rats were sacrificed after they had been on the high fluoride intake for a period of 61 days. At this time the 2 femurs and one incisor were removed from each animal, and again a blood sample was collected. The femurs and incisors were dried, and defatted. Analysis of one of each pair of femurs and the incisors for calcium, magnesium, phosphate, sodium and potassium was carried out(5,9) after heating the samples to constant weight to determine the ash content. The remaining femurs were used for fluoride determination. They were ground with an agate mortar and pestle and a sample (5-10 mg) of the powdered bone used for

* I am indebted to Dr. R. M. Archibald for encouragement during the course of this work. The technical assistance of Mr. Compton de Abreu and Miss Lucia Seccone was invaluable. This study was supported in part by U.S.P.H.S. Grant AM-03347.

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TABLE I. Urinary and Faecal Excretion of Calcium, Magnesium and Phosphate.

Treatment	Calcium excretion (μequivalents/day)		Magnesium excretion (μequivalents/day)		Phosphate excretion (μmoles/day)	
	Urine	Faeces	Urine	Faeces	Urine (inorg. phosphate)	Faeces (total phosphate)
Control	66.3 (48) ± 5.9	1417 (24) ± 47.7	133.4 (48) ± 5.5	110.0 (24) ± 8.7	335.2 (48) ± 8.6	375.2 (24) ± 19.2
.001% F⁻ (12-22 days)	*42.4 (44) ± 4.3	1292 (20) ± 70.7	129.7 (44) ± 3.9	87.8 (20) ± 8.3	323.1 (44) ± 9.5	335.1 (20) ± 17.6
Control	71.2 (48) ± 5.4	1635 (23) ± 73.9	140.4 (48) ± 3.3	109.4 (23) ± 8.2	323.6 (48) ±12.2	429.7 (23) ± 5.5
.035% F⁻ (7-16 days)	†32.4 (44) ± 3.3	1744 (22) ± 66.1	139.9 (44) ± 3.9	101.4 (22) ± 5.4	†520.8 (44) ±19.4	†291.1 (22) ± 15.2

Results are expressed as mean (No. of determinations) ± S.E.M. * $p < .005$ † $p < .001$

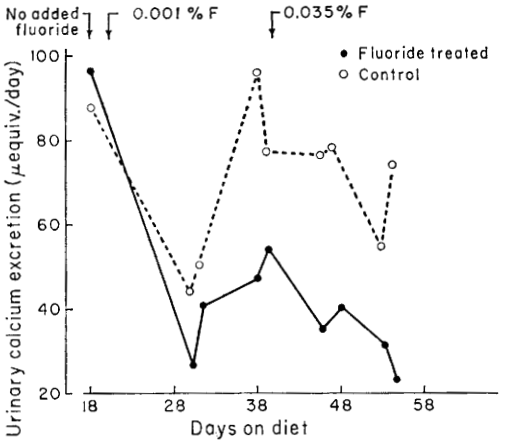

FIG. 1. Effect of sodium fluoride ingestion on urinary excretion of calcium in the rat.

the determination by a modification of the method of Wharton(10). The blood was analyzed for calcium, magnesium and inorganic phosphate. The results were analyzed statistically by the "t" test(11).

Results. The excretion of calcium, magnesium and phosphorus during the balance periods when fluoride was given is compared with that of the controls in Table I. The effect of fluoride at the initial dose of 0.001% on excretion of calcium in the urine is marked (Fig. 1). Further diminution in calcium excretion was observed with the higher doses of fluoride. In addition the higher fluoride dose produced a gross alteration in absorption of phosphate from the gut and also excretion in the urine of inorganic phosphate. The faecal phosphate was diminished and urinary output increased. A rise in plasma inorganic phosphate (Table II) found at the end of the balance periods when F^- intake was 0.035% persisted to the end of the experiment. No alteration in either the calcium or magnesium level in the plasma was found after the high dose of fluoride.

Table III shows the ash content and the sodium, potassium, magnesium, phosphate, calcium and fluoride content of the femurs at the end of the balance periods and also at the end of the experiment (that is after 61 days on the high fluoride intake). The incorporation of fluoride into bone in the animals of Group II is considerably higher at the end of the experiment than after 14 days on

TABLE II. Plasma Calcium, Magnesium and Inorganic Phosphate Following Ingestion of 0.035% F^- in Diet.

Treatment	Plasma calcium (mequiv./l)	Plasma magnesium (mequiv./l)	Plasma inorg. phosphate (mequiv./l)
Control	5.6 (8) ± .03	1.8 (8) ± .03	4.6 (8) ± .02
.035% F^- (16 days)	5.5 (8) ± .01	1.7 (8) ± .07	*5.6 (8) ± .36
Control	5.7 (7) ± .1	1.6 (7) ± .03	4.0 (7) ± .2
.035% F^- (61 days)	5.8 (5) ± .1	1.7 (5) ± .02	†5.2 (5) ± .3

Results are expressed as mean (No. of determinations) ± S.E.M. * $p < .05$ † $p < .005$

the high fluoride intake and shows that the bone has a remarkable capacity for fluoride. There is an increase in magnesium in bone as early as 14 days after commencing the higher fluoride intake. Prolonged exposure to the high fluoride resulted in an increase in bone potassium concentration but this was not statistically significant. There is no alteration in concentration of the other inorganic components measured nor was any change in the ash content of the bones detectable following fluoride ingestion.

In the whole incisors which were analyzed for calcium, magnesium, sodium, potassium, phosphate and ash content, there was a marked increase in both sodium and potassium (Fig. 2). The change in magnesium concentration associated with high fluoride intake was not significant.

Discussion. Small amounts of fluoride ingested by humans—0.5-1.0 mg per day—are excreted(12) while at high levels of intake, increased absorption, storage of fluoride in bone and teeth and an increase in the amount in urine occurs. Ingestion of water containing 8 p.p.m. of fluoride over a period of 30 years has been associated with dental fluorosis with the possibility of increased density of bone(13); by contrast drinking water containing less than 0.1 p.p.m. F^- was associated with a high incidence of osteoporosis. Experimental animals—rats, kittens and rabbits—appear able to tolerate at least 10 times the fluoride intake of humans without adverse effect(14). Although an intake of 10 p.p.m. of fluoride in the drinking water has been reported to be extremely toxic to humans (15) it is not clear whether other factors contributed towards the toxicity, since the high temperatures experienced seemed to accentuate the symptoms and malnutrition may have been a significant accompanying factor.

The initial dose—0.001% F^- in the diet—given rats in the present work represents an average daily intake for a 40-80 g rat drinking 10 ml of water containing 10 p.p.m. The higher dose—0.035% F^-—corresponds to an intake of approximately 100 p.p.m. in the drinking water of the older rats. Slightly diminished weight gain noted by Talmage and Doty(16) when water containing 187 p.p.m. F^- was given to 160-180 g rats may have been related to food intake. In the present work a sudden retardation in weight

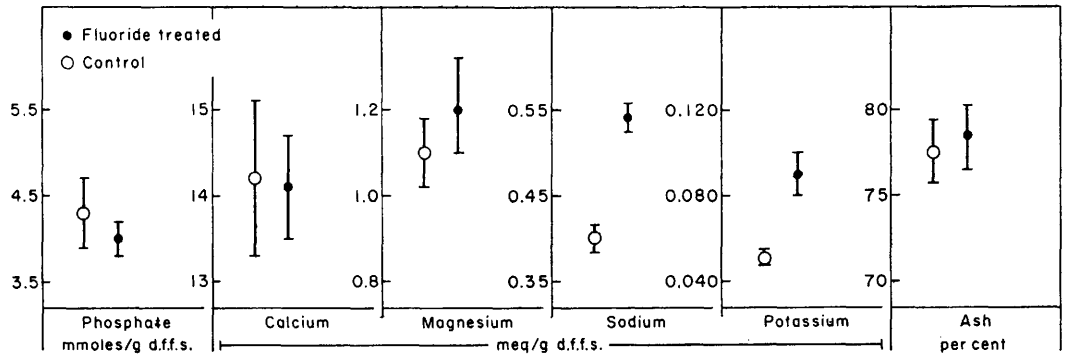

FIG. 2. Electrolyte composition and % ash of whole incisors after administration of 0.035% F for 61 days. Mean values ± S.E.M.

TABLE III. Electrolytes in Bone After Fluoride Administration.

	Calcium (mequiv./g D.F.F.S.)	Magnesium (mequiv./g D.F.F.S.)	Phosphate (mmoles/g D.F.F.S.)	Sodium (mequiv./g D.F.F.S.)	Potassium (mequiv./g D.F.F.S.)	Ash (% D.F.F.S.)	F^- (% of ash)
Control	10.1 (8) ± .14	.280 (8) ± .004	3.3 (8) ± .2	.266 (8) ± .05	.095 (7) ± .005	63.3 (8) ± .7	.038 (8) ± .01
NaF treated .035% F^- (16 days)	10.4 (8) ± .18	*.323 (8) ± .015	3.6 (8) ± .2	.300 (8) ± .07	.104 (8) ± .007	64.9 (8) ± 1.5	† .537 (6) ± .03
Control	10.7 (8) ± .24	.278 (8) ± .003	3.8 (8) ± .1	.304 (8) ± .01	.076 (8) ± .002	67.9 (8) ± 1.3	.083 (5) ± .04
NaF treated .035% F^- (61 days)	10.7 (7) ± .11	*.316 (7) ± .014	3.7 (7) ± .1	.326 (7) ± .01	.086 (7) ± .006	67.1 (7) ± 1.8	†1.07 (6) ± .06

Results are expressed as mean (No. of determinations) ± S.E.M. * $p < .05$ † $p < .001$

gain occurred when the intake of fluoride was increased to 0.035%, but this was followed after 3 days of adaptation by an increase in weight at the same rate as the control animals. Since the animals were tube fed the retardation was not due to a change in food consumption.

The depressed urinary calcium excretion when fluoride intake was low is in agreement with that reported in humans suffering from osteoporosis treated therapeutically with fluoride(4) and may reflect enhanced deposition of calcium in bone. A resulting depressed plasma calcium level undetected by the analytical methods used could be sufficient to produce the differences in calcium excretion observed.

The marked increase in phosphate absorption in the gut and excretion of inorganic phosphate in the urine on the high fluoride dose previously reported(17) raises the question as to whether absorption and excretion of phosphate are interdependent. A primary action by phosphate is suggested by the enhancement of fluoride absorption from the gut in rats in the presence of sodium dihydrogen phosphate(18). Although higher than normal inorganic phosphate has been reported in human patients with fluorosis (19) the data are inconclusive since there was no control over phosphorus intake. Increased inorganic phosphate excretion in monkeys fed fluoride has also been reported (19).

Although Lantz and Smith(20) found that an *ad lib* diet containing 0.05-0.1% sodium fluoride caused an alteration in phosphorus retention by the body, the results were variable. Generally the fluoride treated rats excreted increased phosphate *via* the gut, a finding contrary to that in the present work.

Bone has a tremendous capacity for fluoride. Fourteen days after receiving 0.035% F^- in the diet the ash from the femurs contained 0.5% F^-; the concentration in the growing rats had risen to 1.0% after 61 days.

The increased bone magnesium associated with increased fluoride concentration found here and reported by others(21,22) presents some incompatibility between the postulation that bone magnesium is located on the crystal

surface(23) and that the crystal size increases with increasing fluoride concentration (24).

The marked increase in potassium and sodium concentration in the whole incisors is striking. Analysis of the dentin, enamel and pulp separately is necessary to localize the site of the increase. This warrants further investigation in view of the protective property of fluoride against dental caries(25).

Summary. The ingestion of 0.001% F^- in the diet of young rats decreased the excretion of calcium in the urine. In addition to decreasing the calcium excretion in the urine, 0.035% F^- produced a marked alteration in phosphate metabolism; absorption of phosphate from the gut was increased, plasma inorganic phosphate elevated and its excretion in the urine increased. No change in bone phosphate was found. Apart from concentration of fluoride in bone and an increase in concentration of magnesium in this tissue the mineral composition was the same as in the control animals. Analysis of teeth from the rats after 61 days of high fluoride ingestion revealed a striking rise in the sodium and potassium concentration in the whole tissue.

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Received November 30, 1964. P.S.E.B.M., 1965, v120.