Effects of Sodium and Hydrogen Fluorides on the Metabolism of Fluorine, Calcium, and Phosphorus in Rats

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Rats given 4 mg. fluoride/kg./day for eight days excreted more calcium and phosphorus and retained less than the controls. In bone ash from rats given different fluoride doses for 40 days the calcium and phosphorus were maximal after a total dose of about 250 µg. fluoride/rat, but fell to subnormal levels after higher doses. The calcium and phosphorus levels in bone ash from rats given 750 µg. fluoride/day fell with the duration of treatment.

Rats exposed to 9.4-11.7 µg. hydrogen fluoride/litre air absorbed fluoride rapidly, as shown by increased urinary excretion, by changes in the enamel of the teeth, and by rising fluoride levels in the teeth and bones. Radiological examination, however, showed no gross changes. Urinary excretion increased with exposure time.

Fluorine compounds are widely used in industry, *e.g.*, in aluminium and glass manufacture. The gaseous or solid fluorides released in these processes affect both the manufacturing areas and their surroundings.

There is no general consensus of opinion on the influence of high doses of fluoride on the metabolism of calcium and phosphorus, although most investigators find increased excretion of calcium, especially in the faeces. The interaction of fluoride and calcium ions explains, for instance, why the toxicity of fluorides is increased during growth, lactation, and pregnancy, as calcium requirements are higher during these periods.

In this paper the effects on calcium and phosphorus retention of oral sodium fluoride and of inhaled hydrogen fluoride are presented. The work consisted of the estimation of calcium and phosphorus balance, and of retention by bone, and how it is affected by the duration of administration of fluoride and of the amount administered; and an estimation of the retention of fluorides in bones and incisor teeth after inhalation of hydrogen fluoride.

Materials and Methods

Rats These were of the Wistar strain and weighed 170-250 g. They were fed on the Larsen diet (Fábry, 1955) containing about 6,000 p.p.m. of phosphorus and 11,000 p.p.m. of calcium. The rats were given drinking

water *ad libitum* (distilled in balance studies). They were weighed daily and were kept in metabolic cages, five or six rats to a cage, except in balance studies when they were kept one to a cage.

Effect of Dose Rate In the first series of experiments 77 rats, initial mean weight 170 g., were divided into 11 equal groups. The control group received by probe *per os* 0.2 ml. distilled water and the others received graded doses of sodium fluoride in 0.2 ml. distilled water. The experiment lasted 40 days.

Effect of Duration of Experiment In the second series, 60 rats, initial mean weight 200 g., were divided into four groups. The control group received by probe *per os* 0.2 ml. distilled water. The second, third, and fourth groups received 750 µg. fluoride as sodium fluoride daily for periods of 40, 60, and 140 days respectively. At the end of the experimental period the levels of calcium and phosphorus in the bones were determined.

Balance Studies The rats were kept singly in metabolic cages allowing separate collection of urine and faeces. Eight rats received the standard diet for eight days and then for a further eight days were given orally by probe 4 mg. fluoride/kg. weight and the Larsen diet. The urine and faeces as well as the diet and water were measured daily. At the end of the experiments the rats were decapitated. Calcium was estimated by Urbach's (1934) procedure and phosphorus by Travina's (1955) method.

Inhalation Studies In the inhalation experiment, rats were kept for three hours daily in a chamber flushed

Received for publication July 7, 1967.

with air containing 9.4-11.7 $\mu\text{g./l.}$ of hydrogen fluoride. The rats were divided into four groups, a control group, and groups exposed respectively for 90, 180, and 270 hours. The average temperature was 24.5°C.

After exposure the animals were decapitated and their femurs and tibiae were removed. Fluorides in the urine, bones, and teeth were estimated by Willard and Winter's (1933) procedure, using thorium nitrate, but with methylthymol blue instead of alizarine red as an indicator.

Results

Effect of Fluoride on Calcium and Phosphorus Balance The administration of fluoride retarded the intake of the diet and consequently also of calcium and phosphorus. But in

these circumstances both the faecal and urinary excretion of calcium were higher ($P < 0.05$, Table). The effect of fluoride was also shown by a higher excretion of phosphorus in the urine ($P < 0.01$) and faeces ($P < 0.001$). In both cases there was a much smaller retention ($P < 0.001$).

Effect of Dose Rate and Duration of Administration of Fluoride on Calcium and Phosphorus Levels in Bones The level of calcium in the femur ash rose and then fell as the dose of fluoride was increased (Fig. 1). The highest level was found in rats given 250 $\mu\text{g.}$ fluoride daily (statistically significant, $P < 0.05$). At high doses (2.00-3.00 mg.) it was significantly lower than in the controls ($P < 0.001$).

FIG. 1. Variation of calcium levels in bone ash with fluoride dose. The total doses given over 40 days are shown.

TABLE
CALCIUM AND PHOSPHORUS BALANCE IN RATS GIVEN 4 mg. FLUORIDE/kg./day

Balance (mg.)	Calcium			Phosphorus		
	Larsen Diet	Larsen Diet + Fluoride	Significance	Larsen Diet	Larsen Diet + Fluoride	Significance
No. of rats	8	8	—	8	8	—
Intake	171.70 $\pm$ 1.20	157.70 $\pm$ 0.97	$P < 0.01$	89.80 $\pm$ 0.88	84.96 $\pm$ 1.72	$P < 0.01$
Excretion in						
	105.80 $\pm$ 2.58	121.80 $\pm$ 1.49	$P < 0.05$	49.01 $\pm$ 0.58	54.90 $\pm$ 1.23	$P < 0.001$
	17.10 $\pm$ 0.45	19.00 $\pm$ 0.62	$P < 0.05$	19.53 $\pm$ 0.21	22.20 $\pm$ 0.70	$P < 0.01$
Total	122.90 $\pm$ 2.35	140.80 $\pm$ 1.99	$P < 0.001$	68.54 $\pm$ 0.38	77.10 $\pm$ 1.76	$P < 0.001$
Retention	48.80 $\pm$ 2.36	16.90 $\pm$ 2.14	$P < 0.001$	21.26 $\pm$ 0.37	7.86 $\pm$ 1.81	$P < 0.001$

FIG. 2. Effect of duration of administration on calcium levels in bone ash from rats given 750 µg. fluoride/day.

FIG. 4. Effect of duration of administration on phosphorus levels in bone ash from rats given 750 µg. fluoride/day.

The calcium level in bone ash fell as the number of daily doses of 750 µg. fluoride increased (Fig. 2). The fall was significant by the fortieth day ($P < 0.05$).

The phosphorus content of femur ash varied with the dose similarly to the calcium content (Fig. 3). It varies differently however, with the duration of

dosing (Fig. 4). After 750 µg. fluoride/day the phosphorus levels showed a fall only on the 140th day, and then not a big one ($P < 0.05$).

Inhalation Studies The results are summarized in Figure 5. Urinary excretion of fluoride

FIG. 3. Effect of fluoride dose on phosphorus levels in bone ash. The total doses given over 40 days are shown.

Fig. 5. Average amounts of fluoride in urine, bones, and teeth of rats after inhalation of hydrofluoric acid in the air after 90, 180, and 270 hours.

was always raised ($P < 0.001$) and increased with the duration of exposure. Concentrations in femur ash and teeth also rose ($P < 0.05$).

A typical sign of the absorption of fluorides was the state of the teeth. By 90 hrs. there were changes in the enamel of the upper and lower incisors—a bleaching of the yellow-brown tinge and a formation of white stripes. By 270 hrs. there were uneven incisary surfaces, on the upper incisors dark cavity spots and all along the lower incisors dark stripes. These changes were visible, though of different intensities, in all the exposed animals. The fluoride concentrations in the teeth of exposed rats were, in all groups, significantly higher than in the controls ($P < 0.05$).

Radiographs revealed no changes of osteosclerotic or osteoporotic character.

Discussion

The interactions *in vivo* of fluoride with phosphorus and especially with calcium were reviewed by Harkins, Longenecker, and Sarett (1963) and by Chiemchaisri and Phillips (1963). It is evident that fluoride under different conditions affects differently the metabolism of calcium and phosphorus; and also that some calcium compounds reduce the toxicity of a high fluoride intake (Jackson, 1955; Wagner and Muhler, 1960). The antitoxic action of milk (Ham and Smith, 1954) is explained in this way. Our balance experiments are consistent with these findings. We showed that dosing with fluoride significantly increased the excretion of calcium and phosphorus from the rat (Table).

The different effects on calcium and phosphorus metabolism observed *in vivo* seem contradictory but can often be explained by differences in the species and doses used. Baryševa (1958) showed that the dose affected the incorporation of ^{45}Ca and ^{32}P into the incisor teeth of rats. Incorporation of ^{45}Ca was increased by 3 mg. fluoride/kg. and was unaffected by 5 mg./kg. and incorporation of both ^{45}Ca and ^{32}P was greatly decreased by 20 mg./kg.

Another important factor is the duration of administration. The effect of fluoride on the retention of calcium and phosphorus in bones has been investigated by Lengemann and Comar (1963) and by Menczel, Schraer, Pakis, Posner, and Likins (1962). We found that the calcium and phosphorus content in bone ash depended both on the dose and the duration of application in long-term experiments. Whereas small doses increased the levels (the highest were found after 250 μg . fluoride/day) large doses decreased them significantly. The effects of duration of treatment were more evident in the case of calcium (Fig. 2).

Our inhalation experiments agree with those of Hodge (1964). Fluoride caused the same changes whether given by inhalation or in the diet. Bazille (1935) claimed that fluorides were not absorbed by inhalation, but Gadaskina (1937) proved in experiments on rats and rabbits that inhaled hydrogen fluoride is absorbed in the upper respiratory tract (not affected by concentration or time).

Lazarev (1959) showed that 250 μg . hydrogen fluoride/litre of air caused both a deposition of fluoride in the teeth and hypoplastic changes in the dental tissue. In chronic experiments the amount deposited in the teeth and bones increased with time. Our findings in rats agree with these observations (Fig. 5). Deposition of fluorides in bone has often been observed (Machle, Scott, and Largent, 1942; Lawrenz, Mitchel, and Ruth, 1940; Roholm, 1937). Rost (1937) also noted a greater readiness of

bones to fracture. We could not confirm this finding (unpublished).

According to Mestitzová, Ulrich, and Klůčik (1958), the common values for fluoride are 26 mg.% in teeth ash and 30-70 mg.% in bone ash. Using rats, which inhaled fluorides at 0.69-4.43 $\mu\text{g./l.}$ for 8 months, they found changes similar to ours from the inhalation of 9.39-11.66 $\mu\text{g.}$ hydrogen fluoride/litre air for three hours a day for 27 days.

The analyses for fluorides in the first samples of urine after an exposure of only three days showed raised levels, confirming the opinions of Collings, Fleming, and May (1951) and of Collings, Fleming, May, and Bianconi (1952) concerning the rapidity of absorption of fluorides from the lungs and the rapid excretion in the urine. Although in our experiment the concentrations of fluoride in the urine in different specimens were somewhat variable, the mean concentrations from the four series clearly show an increased excretion with increased duration of exposure.

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