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THE INFLUENCE OF SODIUM FLUORIDE UPON THE COMPOSITION OF TIBIAE OF RATS PARTIALLY RECOVERING FROM RICKETS

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McClure (1) has recently called attention to the need for additional information dealing with the localization of fluorine in teeth and bones and with associative factors involved in its absorption and metabolism. The work of Roholm (2) and Shortt (3) with the human being appeared to support this viewpoint strongly. Roholm (2) found that fluoride may cause either osteomalacia or osteosclerosis and that storage of fluorine in the bodies of female workers in the cryolite industry in Denmark was sometimes sufficiently great that, even after they left the factory, enough fluorine to cause tooth damage was secreted in their milk. Shortt (3) et al. found osteosclerosis associated with 30 to 45 years residence in a mottled enamel area in India.

Using rats as experimental animals McClure and Mitchell (4) reported that 0.0106 and 0.0313 per cent levels of fluorine as the sodium salt did not change the per cent retention of the ingested calcium whereas a level of 0.0623 per cent fluorine lowered the per cent of the ingested calcium that could be retained. Smith and Lantz (5) reported that 0.10 per cent sodium fluoride, in a ration otherwise satisfactory, caused lower values than normal for the ash content of the bones of rats. An increase in the calcium to phosphorus ratio in the bone was found to be the result of an increase in the calcium content accompanying a decrease in the phosphorus content. Lantz and Smith (6) reported that 0.10 per cent of sodium fluoride caused rats to retain much less calcium and less phosphorus than normally. Hauck, Steenbock, and Parsons (7), in a study of the effect of calcium intake upon the calcification of bones during fluorine toxicosis, found that on a diet low or moderate in calcium content the ash content of bones of rats was decreased absolutely and percentagely by fluorine, while with a high calcium rachitogenic ration the ash was definitely increased. No results on fluorine content were reported. In another study these authors (8) found that the effect of sodium fluoride upon teeth, when the fluoride was in a low calcium ration, was reduced by the administration of vitamin D.

Schulz (9) reported evidence that rats on a ration containing a high percentage of calcium and a moderate percentage of

phosphorus resisted the toxic action of moderate doses of fluorides better than rats fed rations containing other combinatons of calcium and phosphorus. With such a ration (high calcium-moderate phosphorus) he found an increase in the amount of calcium in the bones of rats. The inclusion of cod-liver oil in the diet or irradiation of the animals with ultra-violet light appeared to inhibit some of the more severe symptoms of fluorosis. In general, feeding of fluorides caused an increase in the fluoride content of bones of rats.

OBJECT OF PRESENT STUDY

The present experiment was planned for the purpose of determining the qualitative effects of administration of fluoride to rats recovering from rickets previously induced by a high calcium-low phosphorus ration. It seemed desirable to investigate the effect of the fluoride upon bone weights, ash content, line tests and fluorine content under the superimposed condition of healing rickets. Although data on the effects of feeding sodium fluoride and other fluorides in rachitogenic diets are available, no data have been observed which deal with the changes encountered in the healing process when sodium fluoride is administered or with the changes in the quantities of fluorine incorporated in bone during the process of healing.

EXPERIMENTAL PROCEDURE

Rats of weaning age were placed on the Steenbock and Black (10) standard rachitogenic ration until they were distinctly rachitic at the close of a 21 day depletion period. They were then divided at random into four groups and their body weights recorded. The four lots were then given the following rations:

Lot 1. Rachitogenic diet plus 0.1 per cent sodium fluoride plus 0.1 ml. of diluted cod-liver oil as the source of vitamin D, sufficient to give approximately 2+ healing.

Lot 2. Rachitogenic diet alone.

Lot 3. Rachitogenic diet plus the same vitamin D supplement as lot 1.

Lot 4. Rachitogenic diet plus 0.1 per cent sodium fluoride.

The rats receiving vitamin D were given their daily supplement of oil by mouth from an accurate pipette. The feed and fluorine free distilled water were kept before the rats at all times. The experiment was run twice. In the first run the animals were sacrificed on the ninth day of the healing period; in the second run they were sacrificed on the eleventh day. Body

Table 1. The Influence of Recalcification of Rachitic Tibiae Upon the Storage of Fluorine.

Lot Number	Supplement	Average Body Wt. Gms.		Average Line test	Average Wt. of tibia grams	Average Wt. of ash grams	Average percent ash	Average F in tibia gamma	Average percent F in tibia	Average percent F in ash
		Start	End							
1	codliver oil 0.10% NaF	59.1	58	2.63	0.1049	0.0444	42.3	125.25	0.118	0.28
2	none	59.9	66.5	0.19	0.1060	0.0403	38.17	10.63	0.010	0.028
3	codliver oil	60.5	64.1	1.8	0.1046	0.0404* (7 rats)	38.61 (6 rats)	9.73 (6 rats)	0.009 (6 rats)	0.022 (6 rats)
4	0.10% NaF	59.0	60.0	0.75	0.0986	0.0392	38.71	92.36 (7 rats)	0.090 (7 rats)	0.24 (7 rats)

* Averages are for 8 animals except when otherwise indicated.

weights were determined just before the animals were sacrificed. Bones for line tests, ash and fluorine determinations were then obtained.

The bones for line tests were placed in acetone until the tests were performed, while those for ash and fluorine determinations were, after careful cleaning, dried at 105° C. in an electric oven for 6 hours. They were then extracted with alcohol and with ether in a soxhlet extractor for a period of 24 hours for each solvent. After drying to constant weight individual bones were ashed in weighed crucibles in an electric muffle. The ash was then used in the determination of fluorine according to the following procedure based on the method of Willard and Winter (11) as revised by Armstrong (12), Hoskins and Ferris (13), McClure (1), and Lee and Nilson (14).

The entire ash from the tibia was transferred quantitatively to a 50 ml. Claissen flask along with three glass beads, 0.03 gm. powdered silica, 7 ml. distilled water, 7 ml. of 60 per cent perchloric acid and 0.7 ml. of 20 per cent silver perchlorate. After closing the flask and attaching the condenser distillation was begun by heating the Claissen flask gradually with a micro-burner until the contents boiled and the temperature rose to 140° C. at which time steam from an attached generator was passed into the Claissen flask in the manner suggested by Hoskins and Ferris (13). The head of steam and the heating of the Claissen flask were so controlled as to mantain the distillation temperature between 138° C. and 142° C. and to yield 180 ml. of distillate in 70 to 90 minutes. The distillate was collected in a 300 ml. erlenmeyer containing 5 drops of 5 per cent sodium hydroxide and 1 drop of 1 per cent phenolphthalein solution. More sodium hydroxide was added as required to maintain an alkaline reaction to the phenolphthalein during the distillation. The entire distillate was concentrated by evaporation to a volume of 10 ml., transferred quantitatively to a 100 ml. platinum dish and evaporated down to dryness over a steam bath. The residue was taken up with water and transferred to a 10 ml. volumetric flask. After rendering the solution just acid to phenolphthalein with 0.1 N hydrochloric acid the volume was made up to 10 ml. One ml. aliquots were then pipetted into each of six vials 40 mm. deep and with an inside diameter of 11 mm. One drop of 0.05 per cent sodium alizarin sulfonate was then added, followed by 0.1 N and 0.01 N hydrochloric acid until the light orange transition point of the indicator was obtained. One drop of Hoskins-Ferris (13) buffer mixture was added and sufficient distilled water to fill the vials

to a depth of 25 mm. The fluorine was then titrated with 0.01 N thorium nitrate solution, using a Rehburg micro-burette (Lee and Nilson (14). A recovery test for the distillation and titration yielded 71 gamma from a total of 71.25 gamma of fluoride added to the Claissen flask as sodium fluoride.

Results

Table 1 gives the averages of the values obtained for body weights, line tests, weights of tibiae, weights of ash, per cent ash, amounts of fluorine in tibia ash, per cents of fluorine in tibiae and per cents of fluorine in the ash.

The level of fluoride administered was sufficient to retard growth, but insufficient to cause significant loss in weight during the relatively short healing period. Growth retardation did not appear to be alleviated by the quantity of vitamin D supplied.

The average weights of the tibiae were nearly the same in the first three lots of animals. A difference of only 0.30 mg. existed between the averages of lots 1 and 3, while between lots 2 and 3 a difference of only 1.4 mg. was found.

A 10 per cent increase in the average weight of ash and a corresponding increase in per cent ash were observed when sodium fluoride was added at a 0.10 per cent level to the basal ration supplemented with vitamin D. This increase in ash was not noted when the influence of sodium fluoride alone (lot 4) was compared with that of vitamin D alone (lot 3).

The addition of sodium fluoride alone to the rachitogenic ration (lot 4) increased the fluoride content nearly nine fold without appearing significantly to increase the ash content of the tibiae. The administration of vitamin D with 0.10 per cent sodium fluoride augmented the fluorine content in proportion with the augmented ash.

Conclusions

1. The amount of fluoride in rachitic bones was increased when vitamin D was administered along with sodium fluoride as compared with sodium fluoride alone.

2. This increase in fluorine content is proportional to the increase in the ash content.

3. There was an increased calcification of bones as indicated by the line tests and ash determinations when vitamin D was administered to the ration containing fluoride as compared with a similar ration which did not contain fluoride.

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