Development of Fluorine Toxicosis in the Rabbit.* (19511)

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Chronic fluorine toxicosis has been studied in several species including the rat, guinea pig, chicken, rabbit, dairy cow, and human(1-7). It has been shown that small amounts of fluorides cause a retarded growth rate, loss of appetite, and accumulation of fluoride in the bones. Phillips, Hart, and Bohstedt(8) reported that the daily administration to cattle of 2 to 3 mg of fluorine as rock phosphate fluoride per kg of body weight began to show effects after 3 years. A slight loss in weight, loss of appetite and a decline in milk production was evident.

Experimental chronic fluorine poisoning has been reported in the rabbit. Pachaly(9) reported that changes in the maxillary bones occurred in 2 adult rabbits fed several levels of sodium fluoride for extended periods. Largent, Machle, and Ferneau(10) made a study of fluorine poisoning in several mature rabbits fed diets for a period of 16 to 92 days which contained fluorine and they ingested 12 to 50 mg per kg of body weight per day. Microscopic bone changes developed and were presented in detail. The fluoride content of the skeleton greatly increased but no changes were observed on the external structure of the teeth.

It was the purpose of the present study to determine the effect of fluorosis on the growth rate of the young rabbit, a roughage consuming animal, and to determine if there was a similarity of response in this experimental animal to that of the dairy cow fed rock phosphate fluorine.

Experimental. Young rabbits of both sexes weighing approximately 750 g each were distributed and equilibrated to make 8 lots of 5 animals each. They were kept in wire cages on wood shavings and given food and water *ad libitum*. A weigh-back of the left-over food was made daily in order to obtain records of food consumption. The experiment was extended over a period of 4 months. When the experiment was terminated fluorine analyses were made on the tibia and femur from each rabbit by the method of Willard and Winter (11). The control or basal ration consisted of alfalfa 35%, ground oats 25%, soybean oil meal 9.5%, ground yellow corn 24%, wheat middlings 3.5%, salt .5%, and steamed bone meal 2.5%. The fluorides were administered in the form of the rock phosphate borne fluoride or the more soluble sodium salt. The following diets were used:

Lot No.		% fluorine in diet
1	Basal ration or control	.0008
2	" + .625% raw rock phosphate	.022
3	" + 1.25% " " "	.044
4	" + 2.5% " " "	.088
5	" + NaF	.011
6	" + "	.021
7	" + "	.031
8	" + "	.041

These levels of rock phosphate and NaF feeding were based upon previous data obtained with monogastric animals(12). Since NaF fluorine was found to be approximately twice as toxic as the fluorine in rock phosphate it was anticipated that the effects on the animals fed these rations would be roughly paired as follows: Lots 2 and 5, 3 and 6, and 4 and 8. The raw rock phosphate was substituted for a like amount of the steamed bone meal in the basal ration. Sodium fluoride was fed as a direct addition to the basal ration. The bone meal contained by analysis .033% fluorine, while the rock phosphate contained 3.5% fluorine.

Results. The growth rate data for these rabbits are graphically portrayed in Fig. 1. It is clearly evident that the soluble fluoride present as sodium fluoride in the ration of Lots 5 and 6 had very little, if any, effect upon the growth rate of these young rabbits. The fluorine level fed to Lot 2, as rock phosphate fluorine, and Lot 6 as sodium fluoride fluorine are similar in their fluorine content, yet the

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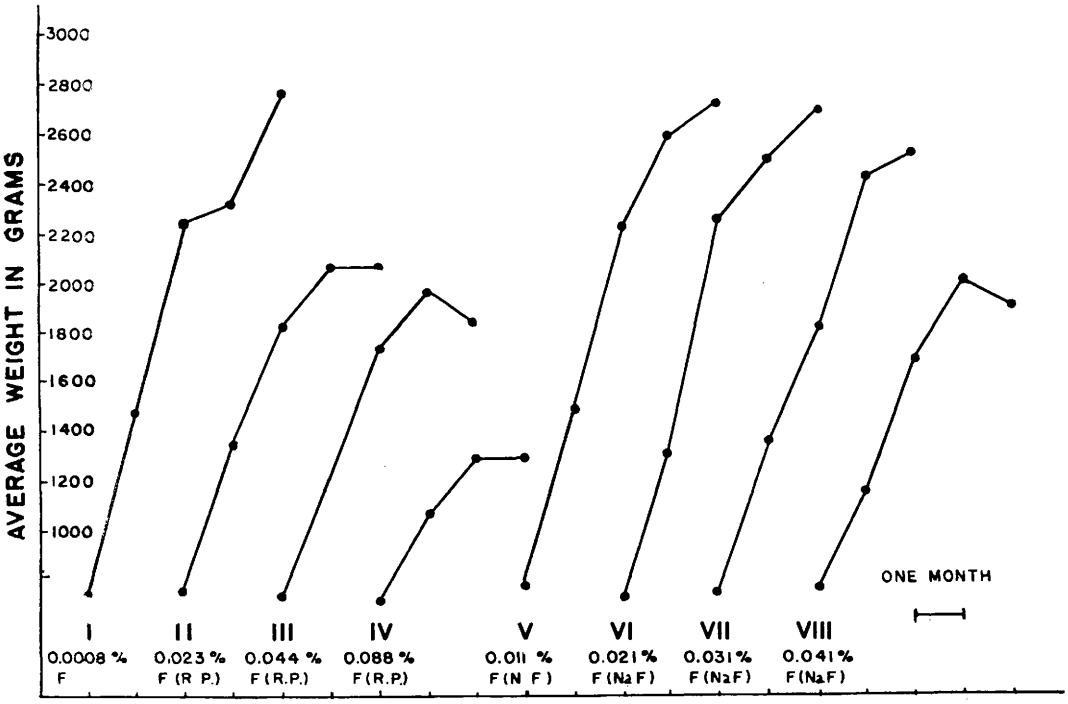

FIG. 1. Effect of various levels of fluorine fed as rock phosphate and as sodium fluoride on the growth of young rabbits.

fluorine in the rock phosphate sharply inhibited growth, while that of the sodium fluoride did not. It was only when the dietary concentration reached .041% fluorine in the form of sodium fluoride that a comparable retardation in growth was obtained equivalent to that of .044% fluorine added as rock phosphate.

Inspection of the data in Table I in part explains the results described above and supports Sollman's appraisal "that fluorine is a protoplasmic poison." The ingestion of fluorine per unit of body weight indicates that the rabbits in Lots 5 and 6 ingested less than 12 mg of fluorine per kg of body weight while those in Lot 2 ingested 13.9 mg of fluorine per kg of body weight. When the ingestion level rose to 16 mg per kg of NaF fluorine there was a lowered average daily gain. The degree of growth retardation in Lots 2 and 7 were alike. These data suggest that .031% fluorine from NaF and .022% fluorine from rock phosphate fluorine are about equal in their effect upon growth. However, .022% fluorine as NaF and .021% fluorine as rock phosphate fluoride caused deposition in the skeleton of nearly equal concentrations, 4360 and 4834 ppm, respectively. The concentration of fluorine in the tibia and femur indicates that the more soluble fluorine of NaF was stored as readily as the rock phosphate fluorine but without an adverse effect upon the growth increment. This fact also may mean that the more soluble sodium fluoride fluorine was ab-

TABLE I. Effect of NaF or Rock Phosphate Fluorine on Growth Increment, Tolerance Levels and Skeletal Fluoride in the Rabbit.

Lot No. and F %		Feed required /100 g gain in body wt, g	Avg daily F ingested, mg	Avg F ingested /day, mg/kg body wt	F content, tibia and femur (dry-fat free), ppm	Avg daily gain in body wt, g
1	Basal	613	.9	.4	275	18.3
2	RP .022	680	22.5	13.9	4360	14.9
3	" .044	765	37.4	23.6	7510	11.1
4	" .088	1090	55.5	50	8938	5.8
5	NaF .011	585	11.7	5.8	3183	18.6
6	" .021	588	21.4	11.3	4834	17.3
7	" .031	680	30.7	16	7075	14.5
8	" .041	735	33.2	22.6	7770	11

sorbed more quickly and deposited in bones much more readily than that of the rock phosphate and hence by this physiologic device did not build up sufficient body concentrations to interfere with growth. At the higher levels the storage was roughly equivalent and growth retardation likewise was equal. Thus, at the low levels of ingestion the skeletal storage was more readily affected by the NaF fluorine and growth was not retarded while rock phosphate fluorine was not stored as readily at the low levels but did retard growth. At the high levels of ingestion the results were much alike in both storage and retardation of growth.

It is apparent from these results that this roughage consuming species was not affected to the same degree as the monogastric animal when the soluble sodium salt was fed as the source of fluorine. This is quite contrary to expectations, and it indicates that the soluble fluoride was not as toxic for the rabbit as it was for the rat when compared to the toxicity of rock phosphate borne fluoride. Rabbits fed the higher levels of fluorine became progressively worse after the first 60 days. This was especially true in Groups 2, 3, 4, and 8, where growth ceased after the third month. The cessation of the growth rate coincided with a very sharply reduced appetite and food intake. At this time these animals were observed to walk with difficulty and exhibited a stiffness in the legs, typical of fluorine toxicosis. The animals receiving .088% fluorine appeared to be in such difficulty as to be practically paralyzed in the hind legs and at the same time became very weak and extremely emaciated.

These data also indicate that since growth in Lots 5 and 6 was not appreciably retarded that the rabbit can withstand 11.3 mg fluorine per kg of body weight per day for the growing rabbit without seriously interfering with the growth process. Apparently increased ingestion above this figure, whether as rock phosphate fluorine or sodium fluoride fluorine, retarded growth in proportion to the increments of fluorine fed. The addition of .022% fluorine (Lot 2) as rock phosphate fluorine resulted in an intake of 22.5 mg per rabbit per day and this amount of fluorine did reduce the rate of growth. This figure then can be taken as being the upper limit of tolerance for the rabbit to rock phosphate fluorine. Further, it is seen that these tolerance levels agree well with the bone storage data obtained with cattle by Phillips, Hart, and Bohstedt (8), in that the bone deposition of fluoride could accumulate without harmful physiologic effect to 5,000 ppm. When the bone fluoride rose to concentrations above 5,000 ppm of fluorine then true fluorine toxicosis developed. On the basis of growth alone it is evident that the minimum toxic level of rock phosphate borne fluorides was below that given to the rabbits in Lot 2. There was less relative difference in the storage between the fluorine of rock phosphate and the sodium fluoride salt, although an increase in the concentration of fluorine in the bones was evident in all lots. Close inspection of these data indicates that proportionately greater storage occurred at the lower levels of fluorine ingestion.

The symptomatology of fluorine toxicosis in the rabbit follows a similar pattern to that observed in cattle. The first evidence of fluorine ingestion was manifest on the surface of the incisor teeth. They lost their pearly, translucent appearance and became white or chalky. This did not affect growth or reproduction at the low levels of fluorine ingestion, but it did at the high levels which were also accompanied by wearing of the molar teeth. This was particularly noticeable in Lots 4, 6, 7, and 8. Gradually, if the levels of fluorine were sufficiently high, stiffness developed due to the exostosis of the leg bones and ankylosis of articulating cartilages. Coincident with this change the bones became a porous, easily penetrable mass in comparison to the compact, tightly formed normal bone.

Summary and conclusions. A study of growing rabbits fed a complete diet supplemented with various levels and forms of fluorine has been made. When levels of fluorine were included in the ration above .021% rabbits in 4 months developed a symptomatology indicating the development of fluorine toxicosis. Growth was retarded, stiffness of the joints occurred, and pronounced changes in the teeth and structure of the bone were encountered. Fluorine was concentrated in the skeleton, and it appears from

these data that the rabbit can withstand a storage of fluorine up to about 5,000 ppm without affecting such physiological processes as growth, health, normal teeth, and bone. In this species the fluorine of raw rock phosphate produced as toxic results, or more so, as an equivalent level of sodium fluoride borne fluorine.

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Anemia of Infection. XVIII. Effects of Turpentine and Colloidal Thorium Dioxide on Rat Plasma Iron Levels.* (19512)

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It was reported previously from this laboratory(1,2) that the intramuscular injection of turpentine into either rats or dogs is followed by transient hypoferremia. It was subsequently demonstrated in dogs that the intravenous injection of a single dose of colloidal thorium dioxide (CTD) is followed by a decrease in the plasma iron, whereas after the administration of three consecutive daily intravenous injections of CTD there is an appreciable increase in the plasma iron above the normal level(3). Following the repeated injection of CTD the hypoferremia-producing effect of turpentine is abolished. Since both iron and CTD are in large part removed from the circulation by the macrophagic tissue, it was suggested that one possible explanation of the above observations is that the injection of turpentine in some way enhances the uptake of iron by these cells and that the repeated administration of CTD temporarily impairs this ability.

The purpose of the present paper is to describe observations dealing with the effect of the intravenous injection of various amounts of CTD and of injections of CTD together with turpentine on the plasma iron of the adult male albino rat.

Methods. Approximately 380 adult male Sprague-Dawley rats weighing 250-300 g were used in these experiments. The diet consisted of Purina dog chow pellets. A 25% suspension of colloidal thorium dioxide (Thorotrast, Heyden Chemical Corporation, New York City, N. Y.) was given intravenously in the amounts indicated in the various experiments. The femoral vein was used for intravenous injections. This vein was surgically exposed under light ether anesthesia and injections were made with a 1 ml tuberculin syringe and a 26 gauge needle. Clean, but not sterile, technic was observed throughout the operative procedures. When hemorrhage occurred as a result of the injections, the animal was discarded from the experiment. The turpentine (0.1 ml/100 g body weight, rectified oil of turpentine, Rexall, USP) was injected into the musculature of the hind leg. The animals were sacrificed by exsanguination

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