

TOXICITY OF SODIUM FLUORIDE IN HAMSTERS

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INTRODUCTION

The development of dental caries in the molar teeth of hamsters is very similar to that in human beings as was first shown by Arnold (1942). The golden hamster, *Cricetus auratus*, and the white rat, *Rattus norvegicus albinus*, are both used extensively in current research on dental caries and periodontal disease. The rather rapid and widespread adoption of the practice of water fluoridation, as well as topical application to the teeth, has more recently stimulated further study of the toxicity of fluoride compounds. The lethal effects of fluorine in large amounts has long been recognized. Dental fluorosis (mottling of the enamel) brought about by the presence of fluorine in drinking water in excess of one to two parts per million likewise has been recognized by dentists for some years. The beneficial effects of fluorine in prevention of tooth decay, when present in drinking water not in excess of 1 to 2 p.p.m., and the prophylactic value of topical application of 2% sodium fluoride have been recent developments. However, according to Shaw (1954), suggestions that the maintenance of teeth was enhanced by the ingestion of fluorides can be found in the literature of the latter part of the nineteenth century. Bunting *et al.* (1928) were among the first to present convincing

evidence of this relationship in their report of a survey of the teeth of school children in Minonk, Illinois, where the water contains 2 to 3 p.p.m. natural fluorides. Hodge (1948) stated that the 2% (20,000 p.p.m.) sodium fluoride used in topical application by dentists is too toxic for other than professional use, that there is little probability that the fluorides used in control of dental caries would or could be crippling (stiffness of the joints, a "poker back", and a gradual loss of motion), and that 1 p.p.m. fluorine addition to the public water supplies probably will result in very mild mottling detectable only by trained experts in *some* children's teeth. Cox and Hodge (1950) reaffirmed these results. The acute toxicity of fluorides in man, reported by Baldwin (1899) and Rabuteau (1867) who took amounts varying from 0.03 gm. to 0.25 gm., was obtained through the employment of more than twice the amount (105 mg.) that could cause death in a 140-pound man (1.65 mg. per kg. body weight reported by Gettler and Ellerbrook, 1939). Shaw (1954) clarified many questions regarding the use of fluorine compounds: what fluorides are; the benefits of ingesting them; the toxicity of these compounds; and safety factors.

The studies here reported on the toxicity of sodium fluoride in ham-

sters were prompted by results of previous experiments on the trichomonocidal value of sodium fluoride (2 to 10,000 p.p.m.) *ad libitum* in hamsters. These animals revealed a rather high tolerance for the compound, and a marked reduction in numbers of trichomonads occurred. This was comparable to the results obtained with carbarsone and a high protein diet (Wantland and Johansen, 1954).

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EXPERIMENTS AND RESULTS

The studies may be grouped into three main series: 1) effects on hamsters receiving *ad libitum* various concentrations (1 to 10,000 p.p.m.) of NaF over a period of several months; 2) effects on hamsters receiving various concentrations (100 to 10,000 p.p.m.) of NaF daily by stomach tube; and 3) attempts to establish precisely the LD₅₀ of NaF for the hamster (amount of NaF in mg. per kg. body weight that kills 50% of experimental animals in 24 hours or less).

An initial study on 20 animals ranging in age from 4 to 8 months involved administering *ad libitum* concentrations of 1 to 8 p.p.m. NaF for a period of 9 months. During this time the animals received a regular diet of pellets and fresh vegetables. Five animals receiving a similar diet and tap water *ad libitum* served as controls. These animals were sacrificed after nine months. No mottling of the teeth was noticed. The animals in nearly every instance

lost weight, but most of them lost only 1 to 8 gm., none more than 13 gm. All organs appeared normal. The consumption of fluoride water was approximately equal to the amount of tap water consumed by the controls.

A second study of 20 older animals (18 months), using concentrations of NaF ranging from 10 to 100 p.p.m. for a period of 7 months *ad libitum*, revealed much the same results, except in degree. The loss of weight was much greater, being from 8 to 36 gm. Nine of these animals died in from 2.5 to 7 months. The strength of the NaF seemed not to be of consequence. Animals on 100 p.p.m. lived the full 7 months and were finally sacrificed. Examination of all animals at autopsy revealed no apparent abnormalities in any of the organs.

A third study of a total of 33 animals, ranging in age from 4.5 to 18 months and in weight from 71 to 179 grams, was carried out giving the NaF *ad libitum* in solutions of 100 to 10,000 p.p.m. In this series results were more pronounced and much faster. Loss of weight was phenomenal. Three animals on 1,000 p.p.m. lost as much as 54% of their original weight in 3 months. The animals on higher concentrations (2,500 to 10,000 p.p.m.) in most cases lost weight at the greatest rate and died within 10 to 15 days. However, 3 animals on 1,000 p.p.m., one on 2,500, and one on 5,000 p.p.m. lived 4.5 months, at which time this particular study was terminated. This study seemed to indicate that the more immediately dangerous concentration was somewhere above 2,500 p.p.m. While half of the ani-

mals on 1,000 p.p.m. died, they did so only after 2.5 to 3 months. The animals receiving 5,000 p.p.m. died, with the one aforementioned exception, in 7 to 14 days. Those animals on 10,000 p.p.m. died within the same time span. All animals receiving 2,500 p.p.m. or more NaF exhibited pronounced lethargy, oedema around the eyes, and a tendency toward softened and sometimes liquid feces. At autopsy the organs of animals in this series appeared normal, except for some hypertrophy of the kidneys and hyperemia and flatulence of the intestine in those given 2,500 to 10,000 p.p.m. NaF.

When it became apparent that NaF in concentrations above 2,500 p.p.m. was lethal to hamsters, sometimes in a matter of days when given *ad libitum*, a fourth study was begun. In this we used a polyethylene stomach tube of 2 mm. bore, a No. 16 hypodermic needle which fit snugly into the tubing, and a syringe to administer the NaF solutions. This enabled us to determine accurately the amount administered daily (0.5 c.c.). Twenty-five animals were utilized in this study: 10 on 10,000 p.p.m. and 3 each on other strengths (100, 500, 1,000, 2,500, 5,000 p.p.m.). Those animals receiving 1,000 p.p.m. or less and one animal receiving 2,500 p.p.m. lived throughout the experiment (6 weeks). Those receiving 10,000 p.p.m. were of special interest because it was believed that this might be very close to the LD₅₀ dose. One half of one cubic centimeter of a 1% solution contains 5 mg. of NaF which would be 50 mg. per kg. body weight in a 100-gram animal. Cox and Hodge (1950) stated that this is the LD₅₀ of NaF for the rat. Later,

however, Shourie *et al.* (1950) stated that the LD₅₀ of NaF for the rat is 80 mg. per kg. body weight. All of the hamsters in this experiment receiving 0.5 c.c. of 10,000 p.p.m. NaF daily by stomach tube died in from 1 to 11 days, with rapid decrease in weight, oedema around the eyes, and general emaciation. The animals on 5,000 p.p.m. and 2 of the 3 on 2,500 p.p.m. lived only slightly longer (11 to 19 days).

In an attempt to establish exactly the LD₅₀ of NaF for the hamster, each of 4 hamsters, after a 24-hour fast, was given 100 mg. per kg. body weight by lightly anesthetizing the animals and inserting the stomach tube down the oesophagus to the stomach. It was almost immediately apparent that all of the animals were nauseated. The respiratory rate was greatly increased (from a normal of 130 it went to 350 per minute) and the amplitude was greatly decreased. Within 10 minutes general coordination was greatly hampered by severe tetany. Violent shaking in the fore part of the animal was noticed, especially the head. Only mild attempts were made by the animals to handle food placed in the cage. This dose turned out to be an LD₁₀₀. Two animals were dead within 3.5 hours, another at 7 hours, and the last one within 12 hours. Autopsy of these animals revealed very fluid intestinal contents and marked flatulence.

In a second attempt to establish the LD₅₀ of NaF for the hamster, the amount given in solution to 4 animals was reduced to 70 mg. per kg. body weight. All four animals became quite ill and exhibited marked lethargy for several hours, but none died.

TABLE 1.—Data Pertinent to Toxicity of Sodium Fluoride in Hamsters.

Dosage, NaF	Method of administration	Number of animals	Age, months	Duration of experiment	Mortality	Average weight loss, grams	
						survivors	non-survivors
1-8 p.p.m.	ad libitum	20	4-8	9 mos.	all lived	5.2
10-100 p.p.m.	ad libitum	20	18	7 mos.	9 died (2.5 to 7 mos.)	10.4	21.0
100-10,000 p.p.m.	ad libitum	33	4.5-18	4.5 mos.	20 died (7 days-3 mos.)	22.0	31.4
100-10,000 p.p.m.	stomach tube 0.5 c.c. daily	25	2-8	6 wks.	15 died (1-19 days)	7.0	35.3
100 mg. per kg. body wt.	stomach tube single dose	4	4	12 hrs.	all died (3.5-12 hrs.)
70 mg. per kg. body wt.	stomach tube single dose	4	11	all lived
80 mg. per kg. body wt.	stomach tube single dose	8	9	4 died (2 hrs.-2 days)	11.5

In a third experiment, each of 8 hamsters was given 80 mg. per kg. body weight. Three of these animals died within 24 hours (at 2, 6, and 9 hours, respectively). A fourth animal died 48 hours after receiving the NaF. Ten minutes after administration there was a marked increase in rate of respiration in all eight animals. The rate was found to reach 350 to 400 per minute. After an hour, breathing settled into a regular, shallow gasping. Oedema around the eyes appeared in 15 minutes. After recovering from the anesthesia (approximately 5 minutes) the animals were quite active for 20 minutes, lethargic for 10 minutes, active again for periods of 5 to 10 minutes, and then again lethargic. Some food was sometimes consumed during the periods of activity. Tetany was not pronounced in animals receiving 80 mg. per kg. body weight, but it was periodically apparent in a small degree. Lethargy became more pronounced at the end of about two hours, and the animals remained quiet until death or until recovery. A summary of the data pertinent to toxicity of sodium fluoride in hamsters is given in Table 1. On the basis of the above evidence, the LD_{50} for NaF in the hamster was concluded to be of the order of 80 mg. per kg. body weight, the same as that found in the study made on rats by Shourie *et al.* (1950).

We have not been able to confirm 80 mg. per kg. of body weight as the LD_{50} of NaF for rats. Two groups of six rats each have survived single doses of 80 to 90 mg. NaF per kg. body weight. These animals did show signs of poisoning, such

as increased respiratory rate and tremor, and in seven animals a pronounced diarrhea was noted about an hour after administration of the NaF. In a third group of 6 rats, each receiving 100 mg. NaF per kg. body weight, 3 animals died within 15 hours. It would seem from the above experiments on rats that the LD_{50} of NaF for this rodent is of the order of 100 mg. NaF per kg. body weight.

Hodge (1948) reported that fluoride probably produces its deadly effects in several ways: namely it is a precipitant of calcium and tends to alter the concentrations of this important physiological ion; it is a powerful enzyme poison and affects a wide variety of enzymes; utilization of sugar is inhibited; blood is rendered uncoagulable through the inhibition of an enzyme (thrombin?); and phosphatases, catalases, and lipases are strongly inhibited. To date we have not checked these reactions to the ingestion of fluoride by the hamster except that we have been unable to confirm the statement regarding the inhibition of the coagulation of the blood. In our experiments, blood from hamsters that had received 80 mg. per kg. body weight formed a solid clot in from 2 to 3 minutes (Sabraze's method). Further study is in progress on the effect on the longevity of hamsters of a daily intake of various concentrations of NaF (2 to 1,000 p.p.m.) in drinking water. Histological studies and chemical analyses of various tissues for fluoride content will be made on these animals.

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