Absorption and Retention of Fluoride from Ingested Stannous Fluoride Dentifrice

By SHARAD S. DESHPANDE and JOHN F. BESTER

Absorption, excretion, and skeletal retention of fluoride were studied in rats fed daily 1.0 mg. of fluoride from stannous fluoride and from a dentifrice containing stannous fluoride. Inhibiting effect of calcium ion accompanying ingested dentifrice upon absorption of fluoride from the intestinal tract was observed. Approximately 50.0 per cent of the ingested fluoride from stannous fluoride and 30.0 per cent of that from the dentifrice were absorbed. There was a steady increase in urinary excretion of fluoride as time progressed. The major portion of absorbed fluoride appeared to be retained in the skeleton. Femurs of rats receiving fluoride from stannous fluoride stored more fluoride than those receiving fluoride from dentifrice. The rats showed a diminishing ability to accumulate fluoride in their skeletons with increase in age.

THE DISCOVERY of the role of fluoride in the prevention of dental caries has initiated considerable research on this element and its metabolism. Stannous fluoride received its first attention when Muhler and Day (1) observed the superiority of this substance over sodium fluoride in protecting rats fed a cariogenic diet. Radike and Muhler (2) showed that a concentration of 10 p.p.m. of stannous fluoride fed to hamsters was roughly twice as effective in preventing caries as the same concentration of sodium fluoride.

Since these early observations there has accumulated incontrovertible evidence that fluoridation of drinking water decreases the incidence of caries. There has also been the introduction of a number of fluoride-containing dentifrices. Neither development has been without controversy; among the areas of dispute have been concern over the quantity of fluoride most desirable and its method of administration. As early as 1956, Hillenbrand (3), speaking for the American Dental Association, pointed to the evidence supporting fluoridation of drinking water as the most effective procedure. A summary of additional evidence was provided in a monograph by Elwell and Easlick (4).

The use of fluoride as an anticariogenic agent is primarily a long term procedure; hence, its possible deleterious effects upon particular patients must be considered. Hillenbrand (3), in referring to a fluoride-containing dentifrice, stated, "It is expected that nearly all of this fluoride will be discharged by rinsing the residual dentifrice from the mouth. Small children, however, might swallow an appreciable portion of the dentifrice, thus contributing to the possibility of dental fluorosis, especially where adequate or excessive levels of fluoride are present in the drinking water." It has been recognized for years that impairment of growth, mottled tooth enamel, and defective bone structure are the chief toxic effects of fluoride in greatly excessive quantities (5).

Very few studies have been made on the retention of fluoride following ingestion of stannous fluoride at high levels. In most of the feeding experiments in rats, sodium fluoride has been used (6–9). The data in all these studies confirm that growth of the skeleton has great influence upon the storage of fluoride in the bones, as young rats retain more fluoride than do old. A retarding effect on growth was observed by Muhler and Day (1) when fluoride was administered to rats at a concentration of 159 p.p.m. Weddle and Muhler (10) gave 2.0 mg. fluoride (as stannous fluoride) to rats by stomach tube daily, for 14 days, and found that 46.0% of the total ingested fluoride was stored in the skeleton. Mitchell (11) reported that from 95.6 to 96.2% of ingested fluoride could be stored in bone, and, in a later report (12) found that continuous feeding of fluoride resulted in a greater retention of the element in bone than did intermittent feeding of the same amount of fluoride. Zipkin (13) found that the concentration of fluoride in bone increased in an essentially linear fashion with increasing concentration of fluoride in drinking water up to 4.0 p.p.m. Largent (14) expressed the opinion that an approximate balance between the intake and output of fluoride is achieved following the establishment of an approximate equilibrium in which the tissues—primarily the skeleton—reach and maintain a certain level of concentration.

Along with skeletal storage, absorbability of fluoride from the gastrointestinal tract must be considered. Among various ions influencing the absorption of fluoride from the tract, calcium would appear to be the most important. An inhibiting effect upon absorption by calcium was observed by Muhler and Weddle (15), by Wagner

Received April 25, 1958, from the School of Pharmacy, University of Southern California, Los Angeles.
Accepted for publication February 25, 1964.
Presented to the Scientific Section, A.PH.A., Los Angeles meeting, April 1958.
Abstracted from a thesis presented by Sharad S. Deshpande, to the Graduate School, University of Southern California, in partial fulfillment of the requirements for the degree of Master of Science.

and Muhler (16), and by Krylova and Gnoevaya (17). The form of inorganic salt accompanying ingested fluoride, its concentration, and its solubility, exert an effect upon absorption of fluoride and upon its storage in the skeleton (18).

All the previous studies of absorption and retention of fluoride from stannous fluoride were conducted on animals receiving the chemical in relatively low concentrations. Because of the possibility of ingestion of dentifrice indicated by Hillenbrand (3) and the apparent lack of knowledge of absorption and retention of fluoride from this source, this study was undertaken. Uptake, excretion, and skeletal retention of fluoride from dentifrice were compared to that of fluoride from stannous fluoride and comparisons made with control rats on a normal diet.

EXPERIMENTAL

A total of 90 rats, Wistar strain, aged 8 weeks was used. The deviation in weight was not more than 5.0 Gm. from the mean. The animals were divided into three groups. Each group contained an equal number of males and females. They were housed in steel cages in groups of five. Feeding cones were used, permitting the animals free access to food. A dentifrice containing stannous fluoride was chosen for the experimental study. As the dentifrice was insoluble in water, it was decided to administer it in food instead of in drinking water. The special food was prepared as follows: 15 Gm. of dentifrice, containing 1.0 mg. fluoride per Gm. made into a thin suspension with distilled water, was evenly mixed with 225.0 Gm. of rat pellet ration. The feed was dried and reweighed to determine the exact amount of dentifrice incorporated. This served as a 3-day supply for each five rats, each rat receiving approximately 1.0 mg. fluoride per 15.0 Gm. of diet. After each 3-day period, any food remaining in the feeding cones was weighed and discarded. In order that the fluoride intake for each five rats remain constant, the amount of fluoride in fresh ration was then increased to compensate for the quantity that was not consumed from the preceding ration. This procedure allowed each rat to receive 1.0 mg. fluoride per day. For the group of rats receiving fluoride supplement in the form of stannous fluoride,[1] 61.9 mg. stannous fluoride (15.0 mg. fluoride) was accurately weighed and dissolved in 25.0 ml. distilled water. The solution was evenly sprayed over 225.0 Gm. of ration and dried. This also was a 3-day supply for five animals. The same procedure for correcting the fluoride intake after each 3-day period was employed. Both of the food supplies were freshly prepared each 3 days because of the instability of stannous fluoride in water. Drinking water for the animals was not restricted. Thus one group of animals, consisting of 15 males and 15 females, each received 1.0 mg. fluoride daily from dentifrice, and a second group of the same number of animals of each sex received a like amount of fluoride daily from stannous fluoride. A third similar group was fed the same diet without fluoride addition.

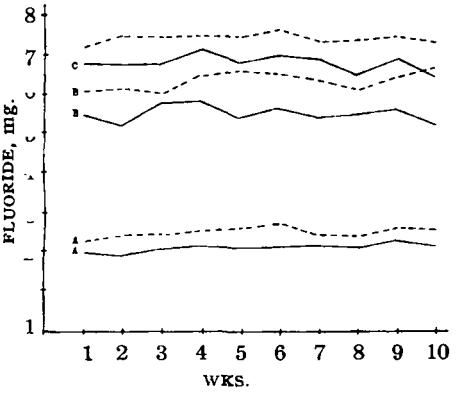

Fig. 1.—Twenty-four-hour fecal excretion of fluoride. (Control animals, *A;* animals receiving stannous fluoride, *B;* animals receiving dentifrice, *C;* solid line, males; broken line, females.)

TABLE I.—PER CENT ABSORPTION OF INGESTED FLUORIDE[a]

Wk.	Sex	Absorbed From—Dentifrice	Absorbed From—Stannous Fluoride
1	M	32.9	51.5
	F	30.0	44.5
2	M	31.5	47.2
	F	28.6	45.8
3	M	33.0	48.6
	F	25.8	45.6
4	M	29.0	48.6
	F	28.6	42.9
5	M	34.3	51.5
	F	30.0	42.8
6	M	30.0	47.3
	F	29.0	44.6
7	M	31.5	51.5
	F	31.5	42.9
8	M	33.0	48.6
	F	27.5	44.6
9	M	30.0	48.6
	F	27.5	41.6
10	M	33.0	51.5
	F	30.0	40.0
Mean	M	31.6 ± 0.55	49.5 ± 0.57
	F	28.9 ± 0.52	43.5 ± 0.58

[a] Total administered less that excreted in feces, expressed as percentage.

Analyses of the diets showed that untreated ration had an average fluoride content of 25.0 p.p.m.; that treated with dentifrice had 91.0 p.p.m.; that treated with stannous fluoride had 93.0 p.p.m.

A metabolism study, similar to that of Weddle and Muhler (19), was carried out on the animals weekly for 10 weeks. Two rats of each sex from each group were taken for each weekly study. Urine and feces, collected over 24 hours, were analyzed for fluoride content.

Skeletal deposition of fluoride was studied by determining fluoride content of the femurs of the rats. The femurs were chosen because they have been shown to have an adequate fluoride content (18). Two rats of each sex from each group were sacrificed every 2 weeks by a blow on the head. The femurs were removed from both legs, cleaned

[1] Obtained from City Chemical Corp., New York, N. Y.

TABLE II.—PER CENT RETENTION OF ABSORBED FLUORIDE[a]

Wk.	Sex	Absorbed from Dentifrice	Absorbed from Stannous Fluoride
1	M	82.9	81.0
	F	84.8	79.4
2	M	81.4	82.0
	F	83.5	79.7
3	M	82.0	79.7
	F	81.9	79.5
4	M	79.0	79.4
	F	82.5	78.0
5	M	82.0	80.5
	F	73.4	74.9
6	M	79.6	88.5
	F	83.4	78.8
7	M	80.0	80.2
	F	83.7	77.4
8	M	80.9	78.9
	F	80.6	78.1
9	M	77.7	78.9
	F	80.6	76.5
10	M	79.6	80.0
	F	81.1	75.4
Mean	M	80.5 ± 0.51	79.9 ± 0.97
	F	81.6 ± 1.00	77.8 ± 0.54

[a] Total absorbed less that excreted in urine, expressed as percentage.

of all extraneous tissue, weighed, and analyzed for fluoride content. The method used for distillation of the fluoride from the samples was that of Willard and Winter (20), with the acceptable modification (19) that calcium oxide was used as fluoride fixative in the preliminary ashing procedure instead of calcium hydroxide. Fluoride in the distillate was determined colorimetrically using a Bausch & Lomb spectronic 20 colorimeter and the method of Revinson and Harley (21). The method depends upon quantitative decolorization of thorium-chrome Azurol S dye complex by fluorine.

RESULTS AND DISCUSSION

The absorption of fluoride from ingested stannous fluoride or from dentifrice was found by comparing the urinary and fecal excretion of fluoride in the appropriate groups of rats. The total excretion of fluoride in feces by the two experimental groups and the control group is shown in Fig. 1. The fecal excretion of fluoride from each of the sources was relatively constant over the 10-week study period. However, the fact that fecal excretion of fluoride between the groups differed indicates a difference in amount and degree of absorption of fluoride from the two sources.

On the basis of knowledge of weekly excretion of fluoride in urine and feces of the two experimental groups, the percentage absorption of ingested fluoride (Table I) and the percentage retention of absorbed fluoride (Table II) were calculated. Table I shows that an average of 68.4% of the ingested fluoride from dentifrice was excreted in the feces of the male rats, while the females excreted 71.1%. This amount of fluoride in the feces may be considered as the unabsorbed part of that ingested. Statistical evaluation of the data in Table I shows that there is a significant difference in the amount of fluoride absorbed by male and female rats (t = 3.66). The same significant difference obtains in capacity to retain fluoride (Table II). In the present studies, the dentifrice fed to the rats contained 42.0% calcium orthophosphate. Each milligram, therefore, of ingested fluoride from the dentifrice was accompanied by 0.42 Gm. calcium orthophosphate, yielding approximately 0.1 Gm. of calcium ion. This level of calcium was probably responsible for the interference with the absorption of fluoride from the intestinal tract (15–17).

It is interesting to note from Table II that approximately 81.0% of the absorbed fluoride from the dentifrice and 78.0 to 80.0% of the absorbed fluoride from the solution of stannous fluoride were retained by the animals. Hence, in spite of lower absorption rates of fluoride from the dentifrice, a major portion of that which was absorbed must have been retained.

Numerous reports (14, 22, 23) on urinary fluoride excretion show that, at a given level of fluoride ingestion over a sufficient period of time, a steady state of equilibrium is established, so that urinary excretion of fluoride essentially equals absorption. The data in Table II show that such a state of equilibrium had not been reached after 10 weeks as fluoride excreted was still only a relatively small percentage of that absorbed. Figure 2 indicates a small, but steady, increase in urinary excretion of fluoride over the study period, indicating that growing rats have a diminishing ability to retain fluoride. This is in agreement with earlier reports (7, 9).

Fluoride balances in the two experimental groups at each weekly interval appear in Table III. High fluoride balance from ingestion of fluoride from solution of stannous fluoride, compared to that from dentifrice, is clearly evident. These positive

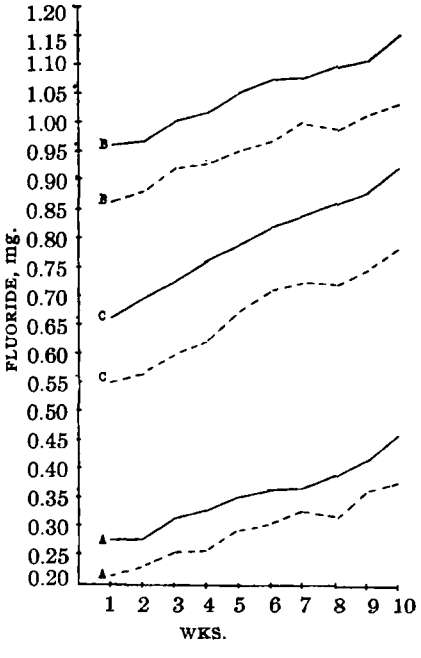

Fig. 2.—Twenty-four-hour urinary excretion of fluoride. (Control animals, *A;* animals receiving stannous fluoride, *B;* animals receiving dentifrice, *C;* solid line, males; broken line, females.)

TABLE III.—FLUORIDE BALANCE IN RATS[a]

Interval, Wk.	Fluoride from Stannous Fluoride Male	Fluoride from Stannous Fluoride Female	Fluoride from Dentifrice Male	Fluoride from Dentifrice Female
1	41.7	35.1	27.3	25.4
2	43.1	36.4	25.5	23.8
3	38.7	36.3	27.5	21.5
4	38.5	35.0	22.6	23.7
5	41.4	32.0	28.1	25.1
6	37.0	35.0	24.0	23.5
7	41.2	33.2	25.1	24.8
8	38.3	34.7	26.4	23.2
9	41.1	31.1	23.4	23.2
10	38.3	30.1	26.1	24.5
Mean	39.9 ± 0.64	34.0 ± 0.70	24.6 ± 0.67	23.8 ± 0.37

[a] All percentages obtained by the following formula:

$$\frac{\text{Amount of fluoride ingested per week} - \text{Amount of fluoride excreted in feces and urine}}{\text{Amount of fluoride ingested per week}} \times 100$$

fluoride balances in both groups of rats confirm previous reports (24, 25).

It is known that retention of absorbed fluoride occurs primarily in the skeleton (10, 11). The retention of this fluoride, represented by accumulation of the element in the femurs, is shown in Table IV and Fig. 3. The amount of fluoride retained in the femurs of the rats receiving a solution of fluoride was definitely at a higher level than that found in rats given fluoride in dentifrice. Although females from both experimental groups retained less fluoride than did males, when these absolute amounts of femur fluoride were converted to parts per million of ashed weight, it became apparent that females retain a higher proportionate amount of fluoride. This is in accord with the findings of Weddle and Muhler (19). It is also evident that the skeleton was still able to accumulate more fluoride, as there is no indication of the femurs having reached saturation levels at the end of 10 weeks

In conclusion, it would appear that the fluoride in stannous fluoride-containing dentifrices is appreciably absorbed from the gastrointestinal tract when the dentifrice is ingested. Accumulation of the element in bone can and does occur and that accumulation continues for weeks if the quantity ingested is sufficient.

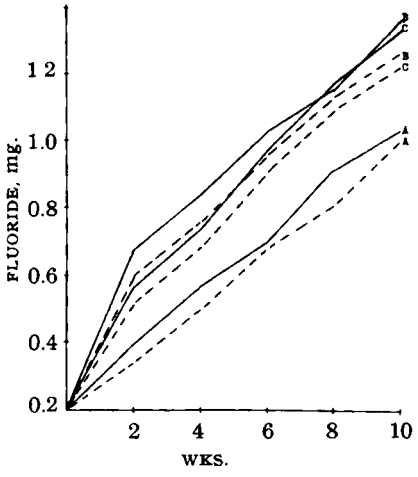

Fig. 3.—Amount of fluoride in both femurs. (Control animals, *A;* animals receiving stannous fluoride, *B;* animals receiving dentifrice, *C;* solid line, males; broken line, females.)

TABLE IV.—DEPOSITION OF FLUORIDE IN FEMURS OF RATS

Wk.	Group	Sex	Dry Wt. of Femurs, Gm.	Ashed Wt. of Femurs, Gm.	Fluoride Content, mg.	Fluoride, mg./Gm., Ashed Wt.
2	Control	M	0.482	0.243	0.190	0.79
		F	0.369	0.185	0.150	0.83
	Stannous fluoride	M	0.476	0.247	0.475	1.90
		F	0.354	0.184	0.400	2.20
	Dentifrice	M	0.481	0.243	0.375	1.54
		F	0.383	0.202	0.320	1.60
4	Control	M	0.719	0.368	0.370	1.02
		F	0.523	0.269	0.310	1.10
	Stannous fluoride	M	0.726	0.362	0.640	1.80
		F	0.510	0.250	0.560	2.20
	Dentifrice	M	0.792	0.363	0.550	1.50
		F	0.512	0.262	0.500	1.90
6	Control	M	0.849	0.437	0.500	1.10
		F	0.603	0.317	0.490	1.50
	Stannous fluoride	M	0.863	0.447	0.830	1.80
		F	0.634	0.326	0.760	2.30
	Dentifrice	M	0.852	0.430	0.770	1.70
		F	0.623	0.310	0.720	2.30
8	Control	M	0.977	0.494	0.710	1.40
		F	0.765	0.397	0.620	1.60
	Stannous fluoride	M	1.023	0.513	0.960	1.80
		F	0.776	0.397	0.940	2.40
	Dentifrice	M	0.952	0.482	0.970	2.02
		F	0.732	0.357	0.900	2.50
10	Control	M	1.438	0.720	0.920	1.29
		F	0.978	0.491	0.800	2.20
	Stannous fluoride	M	1.369	0.713	1.160	1.60
		F	0.935	0.480	1.060	2.20
	Dentifrice	M	1.423	0.708	1.140	1.50
		F	0.912	0.454	1.020	2.20

REFERENCES

(1) Muhler, J. C., and Day, H. G., *J. Nutr.*, **447**, 413 (1951).
(2) Radike, A. W., and Muhler, J. C., *J. Dental Res.*, **32**, 807(1951).
(3) Hillenbrand, H., *J. Am. Dental Assoc.*, **52**, 369(1956).
(4) Elwell, K. R., and Easlick, K. A., "Classification and Appraisal of Objections to Fluoridation," The University of Michigan, Ann Arbor, 1960.
(5) Floyd, D., *Medicine*, **12**, 1(1933).
(6) Glock, G. E., Lowater, F., and Murray, M. M., *Biochem. J.*, **35**, 1235(1941).
(7) Zipkin, I., and McClure, F. J., *J. Nutr.*, **47**, 611 (1952).
(8) Miller, R. F., and Phillips, P. H., *ibid.*, **59**, 425 (1956).
(9) Savchuck, W. B., and Armstrong, W. D., *J. Biol. Chem.*, **193**, 575(1951).
(10) Weddle, D. A., Muhler, J. C., and Stewart, J. O., *J. Dental Res.*, **34**, 895(1955).
(11) Mitchell, H. H., *J. Nutr.*, **19**, 536(1940).
(12) *Ibid.*, **20**, 389(1940).
(13) Zipkin, I., *A.M.A. Arch. Ind. Health*, **21**, 329(1960).
(14) Largent, E. J., *ibid.*, **21**, 318(1960).
(15) Muhler, J. C., and Weddle, D. A., *J. Nutr.*, **55**, 347 (1955).
(16) Wagner, M. J., and Muhler, J. C., *J. Dental Res.*, **39**, 49(1960).
(17) Krylova, M. I., and Gnoevaya, V. L., *Vopr. Pitaniya*, **15**, 37(1956).
(18) Donovan, A., Weddle, D. A., and Muhler, J. C., *J. Nutr.*, **54**, 437(1954).
(19) Weddle, D. A., and Muhler, J. C., *J. Dental Res.*, **34**, 900(1955).
(20) "Official Methods of Analysis," 8th ed., Association of Official Agricultural Chemists, 1955, p. 415.
(21) Revinson, D., and Harley, J. H., *Anal. Chem.*, **25**, 794(1953).
(22) Schweinsberger, R. A., and Muhler, J. C., *J. Dental Res.*, **35**, 760(1956).
(23) McClure, F. J., Mitchell, H. H., Hamilton, T. S., and Kinser, C. A., *J. Ind. Hyg. Toxicol.*, **27**, 159(1945).
(24) Machle, W., and Largent, E. J., *ibid.*, **25**, 112(1943).
(25) Ham, M. P., and Smith, M. D., *J. Nutr.*, **53**, 215 (1954).

Antitussive Action of Aerosolized Terpenes in Guinea Pigs and Humans

By S. J. DESALVA, R. A. EVANS, and R. B. BUTLER

The present study has established with both animal and human assays that the use of aerosolized terpenes does produce significant antitussive effects, and these effects are related to the concentration of the materials in the exposed atmosphere. Moreover, it would appear that the effectiveness of this type of therapy is related to an action on the smooth muscles of the bronchi and bronchioles.

INHALATION THERAPY, *e.g.*, vaporizers, mist, and aerosols, for the treatment of respiratory disturbances has been accepted as a reasonable procedure (1). This is particularly evident in the legendary use of volatile oils for the relief of coughs. The basis for this type of treatment has been mainly empirical. It appeared pertinent at this time to undertake an investigation of the antitussive effectiveness of certain of these volatile substances, given as aerosols,[1] with controlled conditions such as the ammonia-induced cough assay in guinea pigs (2) and the citric acid-induced cough assay in humans (3). This report is a summary of our findings which offers to the reader animal and human data obtained through the efforts of the same investigators.

EXPERIMENTAL

Antitussive Assays in Guinea Pigs

Method.—The method employed is a modification of that described by Winters and Flataker (2). Test animals (350 to 450 Gm. body weight) were individually placed for 1 minute in a 1-L. exposure chamber—a plastic cylinder—into which ammonia vapors were introduced. Vapors were delivered at 10 p.s.i. from an Ohio nebulizer, which contained 200 ml. of 0.5% ammonia water (Merck 28% stock) in the reservoir. The concentration of ammonia in the exposure chamber was relatively constant under the experimental conditions used.

Ammonia-induced coughs were recorded on a paper oscillograph (Grass model 5) *via* a pressure transducer (PT 5-A) which was connected to the exposure chamber and the oscillograph. The transducer was capable of recording respiratory rate as well as body movements and by proper manipulation of the sensitivity gain of the preamplifier, the gross body movement measurements could be made less apparent. Prior to the antitussive evaluation, the animals were screened to determine their susceptibility to ammonia vapors. Animals that coughed at a frequency of between 10–40 coughs per minute were selected for the drug study which was generally performed at least 2 hours afterwards. In any particular group, the test animals were selected in a manner such that their cough frequency response did not vary more than 10 per minute. This arbitrary selection helped to diminish wide variability within any test group. For the most part, the variations in mean cough frequency response among the test groups were within the range of 10 coughs per minute.

To facilitate the statistical processing of the data, the changes in cough frequency responses between pretreatment and treatment measurements were converted to percentiles of pretreatment values. Generally, five animals per test level sufficed but in some instances, it was necessary to increase this number. Throughout the animal study a control nontreated group of animals was always evaluated simultaneously with treated animals. This monitoring was performed in order to eliminate any variable within the laboratory which might influence the assay and could not otherwise be detected.

Received September 25, 1963, from the Colgate-Palmolive Research Center, Research and Development, New Brunswick, N. J.
Accepted for publication November 7, 1963
[1] Marketed as Congestaid by the Colgate Pharmaceutical Laboratories.