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Further Studies on Fluoride Absorption.* (28895)

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Fluoride absorption has been studied by several different approaches including isotope studies with labeled fluoride(1-3), metabolism studies utilizing urinary and fecal fluoride levels in comparison to skeletal retention values(4-7), and by using isolated intestinal segments in experimental animals(8-11). Earlier studies in our laboratories employing the latter technique confirmed other reports as to the rapid rate of fluoride absorption and further indicated that, in the rat, fluoride is absorbed not only in the stomach but also in the duodenum and small intestine. The present studies were conducted using the isolated intestinal segment technique to determine what effect anesthesia, starvation, ascorbic acid, and different inorganic ions have upon rate of fluoride absorption in the rat.

Experimental. Series I was designed to measure the rate of fluoride absorption from the entire length of the gastrointestinal tract of the albino rat. A total of 60 male albino Sprague-Dawley strain rats, ranging in weight from 70 to 90 g, were divided equally into 10 groups. The animals were starved for 30 hours before administration of fluoride. Each animal was given 1.0 mg of aqueous fluoride (as sodium fluoride) by means of a stomach tube. At 10 different periods following fluoride administration the animals were sacrificed and the entire gastrointestinal tract removed by the technique previously described(11). The tracts were placed in silica dishes, covered with exactly 1.0 g of fluor-

ide-free calcium oxide, slurried with redistilled fluoride-free water, evaporated to dryness under infra-red lamps, and ashed in a muffle furnace at 600°C for 6 hours. Aliquots of the weighed ash were then analyzed for fluoride by perchloric acid distillation and titration of the distillate with thorium nitrate as described previously(12).

Series II was designed to study the effect of anesthesia upon rate of fluoride absorption. Eighteen female Sprague-Dawley strain albino rats weighing between 100 and 130 g were divided equally by weight into 3 groups. All of the animals were starved 24 hours prior to the study and were then given 1.0 mg of fluoride (as sodium fluoride) using stomach tube techniques. Animals in Group 1 were anesthetized with ether while those in Group 2 were anesthetized with sodium nembutal. Animals in Group 3 served as controls and were not anesthetized after fluoride administration. After a 2-hour absorption period, all animals were sacrificed and their entire gastrointestinal tracts ligated at the esophagus and rectum, and fluoride content determined as previously described.

Series III was designed to measure the effect of starvation upon fluoride absorption. Twenty Sprague-Dawley strain albino rats weighing between 70 and 90 g were divided equally into 2 groups. Group 1 was starved for 24 hours prior to administration of fluoride while Group 2 received a low-fluoride (0.8 $\mu\text{g/g}$) stock corn diet and distilled water *ad libitum*. Immediately after administration of 1.0 mg of aqueous fluoride (as sodium

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fluoride) by stomach tube to each group, 4 animals from each group were sacrificed and the fluoride content of the entire gastrointestinal tracts determined. Two hours later the entire gastrointestinal tracts from the remaining animals in each group were removed and analyzed similarly.

Series IV was designed to evaluate the effect different inorganic ions have upon fluoride absorption. Thirty-six Sprague-Dawley strain albino rats weighing between 110-140 g were divided equally according to body weight into 6 groups. The rats were starved for 24 hours and then given by stomach tube 1.0 mg of fluoride (as sodium fluoride) mixed with the inorganic ions under investigation. Animals in Group 1 received only fluoride, while the animals in Group 2 received 1.0 mg of calcium (as calcium chloride). Groups 3 and 4 received 1.0 mg of magnesium and iron (as magnesium chloride and ferric chloride), respectively, while those in Groups 5, 6, and 7 received 1.0 mg of phosphorus, sulfate, or molybdenum, respectively, as sodium dihydrogen phosphate, sodium sulfate, or ammonium molybdate. After a 4-hour absorption period the animals were sacrificed, their entire gastrointestinal tracts ligated, removed, and analyzed for fluoride.

Series V was conducted to further study the effect of molybdenum upon fluoride absorption. In contrast to Series IV, adult animals were used in this series. Twelve adult male albino rats from our own breeding colony were divided into 2 equal groups and starved for 36 hours. Each animal in the first group was given 1.0 mg of fluoride as aqueous sodium fluoride and the second group received in addition to the fluoride 1.0 mg of molybdenum as ammonium molybdate. After 2 hours the entire gastrointestinal tracts were removed and analyzed for fluoride.

The last portion of this study (Series VI) was designed to study the effect of ascorbic acid upon rate of fluoride absorption in guinea pigs. Thirty male guinea pigs ranging in weight from 340 to 420 g were divided by weight into 2 groups and starved for 24 hours. The animals were anesthetized lightly with ether to allow intubation and were given 1.0 mg of fluoride (as sodium fluoride) by

TABLE I. Rate of Fluoride Absorption from Gastrointestinal Tract of the Rat as a Function of Time Following Administration of 1.0 mg Fluoride (as NaF) at Time Zero. (Series I).

Time after F adminis- tration (hr)	Avg % F absorbed	Time after F adminis- tration (hr)	Avg % F absorbed
0	—	6	81.3
1	29.1	8	86.4
2	58.1	12	89.5
3	68.5	16	90.8
4	73.4	24	94.9

stomach tube. In addition, the animals in the second group were given 100 mg of ascorbic acid in a similar manner. After periods of 2, 4, and 12 hours, 5 animals from each group were sacrificed, their entire gastrointestinal tracts removed and analyzed for fluoride as previously described.

Data. The results of fluoride absorption as a function of time (Series I) are seen in Table I and are self-explanatory.

The results obtained from the rats in Series II in which the effect of anesthesia on fluoride absorption was evaluated indicate that neither form of anesthesia produced any significant changes in rate of fluoride absorption.

The data pertaining to the effect of starvation upon absorption of fluoride indicate that the gastrointestinal tracts from the animals in Group 1 which were starved 24 hours prior to ingestion of fluoride contained 484 μg of fluoride 2 hours after ingestion indicating that 51.7% of the fluoride had been absorbed. The gastrointestinal tracts from the animals in Group 2 which were not without food at any time during the study contained an average of 609 μg of fluoride indicating that 39.2% of the fluoride had been absorbed. Starvation of the rats for a period of 24 hours prior to ingestion of the fluoride thus resulted in a 32% increase in the amount of fluoride absorbed within 2 hours after ingestion.

The results obtained in Series IV in which the effect of several different inorganic ions upon fluoride absorption was determined are shown in Table II and are self-explanatory. The results obtained in Series V, in which the effect of molybdenum upon fluoride absorption in adult rats was studied, indicate that

TABLE II. Effect of 6 Different Inorganic Ions upon F Absorption 4 Hours After Intubation of Salt Solution. (Series IV).

Group	Ions*	Avg net % absorbed
1	1.0 mg F	52.8
2	<i>Idem</i> + 1.0 mg Ca	36.2
3	" + 1.0 mg Mg	42.0
4	" + 1.0 mg Fe	65.4
5	" + 1.0 mg P	77.4
6	" + 1.0 mg SO ₄	64.9
7	" + 1.0 mg Mo	54.8

* F as NaF; Ca as CaCl₂; Mg as MgCl₂ · 6H₂O; Fe as FeCl₃ · 6H₂O; P as NaH₂PO₄ · H₂O; SO₄ as Na₂SO₄; and Mo as (NH₄)₆Mo₇O₂₄ · 4H₂O.

the animals in Group 1, which received only fluoride, absorbed 51.2% of the ingested fluoride while those animals which received an additional 1 mg of molybdenum absorbed 63.5% fluoride. These data confirm the findings of earlier studies that molybdenum increased the rate of fluoride absorption in adult rats but not in young animals.

The results obtained in Series VI, which concerns the effect of ascorbic acid upon fluoride absorption in guinea pigs, are shown in Table III. These data are self-explanatory and indicate that only after 12 hours were any differences noted in rate of fluoride absorption.

Discussion. A great variety of factors may alter the rate and amount of fluoride absorption. In this series of studies an attempt has been made to evaluate the role of a number of such factors in altering the absorption of fluoride from the gastrointestinal tracts of laboratory animals.

The results again confirm the rapid rate of fluoride absorption from the gastrointestinal tract. It has been shown that complete anesthesia during the fluoride absorption period does not appreciably alter the rate of fluoride absorption indicating that while anesthesia alters other biological processes, it has no apparent effect upon absorption of fluoride in the rat. The absence of normal dietary constituents from the gastrointestinal tract results in a 32% increase in rate of fluoride absorption suggesting that the normal dietary constituents may profoundly retard or even interfere with the absorption of the fluoride ion in the rat. This confirms previous work (12,13) which similarly suggested that food

interferes with fluoride absorption and skeletal retention.

A number of inorganic ions found in normal diets were evaluated for their influence upon rate of fluoride absorption. These data indicate that while calcium and magnesium significantly retard the rate of fluoride absorption, iron, phosphorus, and sulfate increase the amount of fluoride absorbed during a 4-hour test period. Molybdenum produced a variable effect upon fluoride absorption confirming earlier studies in which molybdenum had no effect upon fluoride absorption in young rats(11) although it significantly increased the absorption of fluoride in adult rats(11). In guinea pigs, ascorbic acid may increase the rate of fluoride absorption, especially during the latter stages of absorption, apparently by acting in the duodenum or small intestine with little effect being noted during the first 4 hours of fluoride absorption.

The results of these studies indicate that normal dietary constituents may profoundly alter the absorption of fluoride from the gastrointestinal tracts of laboratory animals. This should be considered in the use of such substitutes for communal fluoridation as fluoride tablets. For example, one might infer that the consumption of a fluoride tablet would not be as effective if consumed immediately following a meal, as it would be if taken at a time when no food is present in the stomach, or that fluoride taken in combination with dietary mineral supplements might have a reduced biological effectiveness since most of these supplements are high in calcium and magnesium and low in phosphorus.

Summary. Studies were conducted to de-

TABLE III. Effect of Ascorbic Acid upon Fluoride Absorption in Guinea Pigs. (Series VI).

Group	Treatment	Time after F admin (hr)	Avg % F absorbed
1	1.0 mg F	2	45.1
		4	50.0
		12	64.3
2	<i>Idem</i> + 100 mg ascorbic acid	2	48.2
		4	52.9
		12	75.4

termine the effect of various different factors upon absorption of fluoride. The results confirm earlier reports concerning rate of fluoride absorption from the gastrointestinal tract with 29.1, 58.1 and 86.4% absorption occurring after periods of 1, 2, and 8 hours, respectively. These data also indicate that anesthesia by ether or sodium nembutal only slightly decreases the rate of fluoride absorption. Starvation prior to ingestion of fluoride increased the absorption of fluoride by 32%. Rate of fluoride absorption in young rats was significantly decreased by calcium and magnesium and significantly increased by iron, sulfate and phosphorus. Molybdenum had no effect in young rats but significantly increased the rate of fluoride absorption in adult animals. Ascorbic acid had only a slight effect upon fluoride absorption in guinea pigs during the first 4 hours after ingestion but increased the absorption after 12 hours.

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In vitro Studies Concerning Fluoride Absorption.* (28896)

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Information concerning the rate of fluoride absorption has been obtained through metabolism data(1,2,3), by using isotopically labelled fluoride(4,5,6), and surgical isolation of intestinal segments in intact animals (7,8,9). However, none of these techniques have produced convincing evidence concerning the actual mechanism by which fluoride is absorbed. For this reason the following series of studies was conducted.

Experimental. The experimental procedure of these studies was similar to that employed by Wilson and Crane(10,11) in studying absorption of carbohydrates from the intestine. An animal was anesthetized by ether inhalation and the desired segment of the gastrointestinal tract quickly and carefully removed. The segment was then everted, ligated at the distal end, and the open proximal end was

attached and tied to a piece of glass tubing, one end of which was drawn out to receive the intestinal segment. The tubing and the intestinal segment were placed in a test tube containing the media under investigation. The system is designed in such a manner that samples may be obtained from the media or from the lumen of the intestinal segment.

In our initial series of studies 40 ml of Ringer's solution containing the desired concentration of fluoride (as NaF) were placed in the outside test tube and 3 ml placed within the lumen of the intestine. The system was maintained at 37°C in a constant temperature water bath under aerobic conditions. At 30-minute intervals aliquots were withdrawn from both inside and outside the intestinal wall. These aliquots were placed in fused silica dishes to which exactly 1.0 g of fluoride-free calcium oxide was added as a fluoride fixative. The samples were evapo-

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