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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TABLE I. Diffusion of Fluoride from Rat Intestine as a Function of Time.

Total μg F added to lumen of intestine	No. trials	% of fluoride disappearing from lumen as a function of time		
		30 min	60 min	90 min
200	7	23.7 ± 2.2*	47.5 ± 2.5	59.4 ± 4.2
400	5	30.8 ± 3.3	53.3 ± 3.0	69.0 ± 1.8
600	5	32.4 ± 2.8	56.8 ± 2.8	68.3 ± 3.0
1000	7	30.3 ± 2.8	52.7 ± 2.1	67.4 ± 2.1

* Standard % error.

rated to dryness under infra-red lamps and ashed for 6 hours at 600°C in a muffle furnace. The fluoride content of each sample was determined by means of perchloric acid distillation and titration of the distillate using acidified thorium nitrate and alizarin as previously described(12).

The intestinal segments used in these studies were removed from Sprague-Dawley strain rats weighing between 120 and 150 g, all of whom were maintained on a low-fluoride (F = 0.05 μg/g) stock corn diet and fluorine-free distilled water.

Data. The data obtained in the initial series of studies indicate that concentrations of 15 or 30 ppm F in the lumen and the outside of either intestinal segments or the stomach remained essentially unchanged for one hour, indicating no active transport of fluoride.

The data shown in Table I indicate the rate of fluoride diffusion from the lumen of rat intestinal segments at 4 different fluoride concentrations. After 30 minutes, 23.7 to 32.4% of the fluoride had diffused from the intestine, and after 60 minutes or 90 minutes, 47.4 to 56.8% and 59.4 to 69.0%, respectively, of the fluoride had diffused from the intestine.

The data in Table II indicate the rate of diffusion of fluoride from the stomach at 2 different fluoride concentrations. After 30 minutes, 14.8 and 16.9% of the fluoride had diffused from the stomach when the amount of fluoride added was 200 and 1000 μg, respectively. After 60 minutes, 25.7 and 28.3% of the fluoride had passed through the stomach wall, while values of 35.4 and 37.1% were obtained after 90 minutes.

The data obtained in the studies designed to show the effect of intestinal length upon rate of diffusion of fluoride from the segments indicate that a direct relationship between rate of fluoride diffusion and the diffusion area was observed, as was to be expected. A series of segments which averaged 4.4 cm in length allowed 12.5 and 22.6% of the fluoride to diffuse from the intestine after 30 and 60 minutes, respectively. When the average segment length was increased to 10.8 cm and initial fluoride concentration held constant, values of 26.1 and 48.5% were obtained during the same time intervals. Employing a fluoride concentration of 200 μg, segments averaging 4.1 cm in length allowed 7.3 and 15.1% of the fluoride to diffuse into the surrounding media. When the average segment length was increased to 8.9 cm in length, the amount of diffusion during the same time intervals increased to 13.6 and 24.9%. These data suggest a direct relationship between the area and rate of fluoride diffusion.

Studies concerning effect of temperature upon rate of the diffusion of fluoride from the intestine indicated that when a concentration of 200 μg of fluoride was added to the lumen of the rat intestine and diffusion studies were conducted at 20, 30, and 37°C, only slight

TABLE II. Diffusion of Fluoride from the Stomach as a Function of Time.

Total μg F added to lumen of stomach	No. trials	% of fluoride disappearing from lumen as a function of time		
		30 min	60 min	90 min
200	6	14.8 ± 5.2*	25.7 ± 3.6	35.4 ± 3.6
1000	5	16.9 ± 3.2	28.3 ± 3.0	37.1 ± 3.3

* Standard % error.

differences in the diffusion rate were noted, indicating that alterations in temperature have very little, if any, influence upon rate of diffusion of fluoride from the intestine. The fact that fluoride diffusion is not temperature dependent is in line with the earlier suggestion that there is no active transport of fluoride through the intestinal wall. This view is also supported by data obtained in a study concerning the effect of enzyme poisons on fluoride diffusion. These data indicate that neither sodium cyanide, sodium iodoacetate, nor 2,4-dinitrophenol alter to any appreciable extent the rate of fluoride diffusion from the inside to the outside of the intestinal segments.

Addition of either calcium or magnesium to the lumen of the intestine was found to decrease significantly the rate of diffusion of fluoride through the intestinal wall. When calcium was added to the isotonic media outside the intestinal wall a normal value for the rate of diffusion was found while similar addition of magnesium was found to increase the rate of fluoride diffusion.

Discussion. These data suggest that neither the stomach nor the intestine of the rat appears to possess a system for active transport of fluoride from the lumen to the circulating body fluids. If such a system were present and operating, one would have expected the concentration of fluoride in the lumen in the reversed system studied to increase as a function of time at the expense of the fluoride on the outside of the gastrointestinal segment. However, the data indicate that when equal concentrations of fluoride were placed in the lumen and in the media outside the intestinal wall no changes in fluoride concentrations occurred during a 60-minute period.

When 4 different levels of fluoride were placed inside the intestinal segments and rate of diffusion of fluoride through the intestinal wall was measured, comparable values were obtained, thus suggesting that the amount of fluoride present is not a major factor governing the initial rate of diffusion. The values obtained for diffusion are somewhat higher than earlier data obtained by a different technique from our laboratories(9). In these earlier studies about 60% of the fluoride was absorbed after 2 hours. However, it has been shown that the age of the rat and his previous exposure to fluoride profoundly alters the rate of fluoride absorption, thus accounting in large measures for these differences.

These data corroborate our previous work, the results of which were obtained by different experimental techniques(9), which show that fluoride is absorbed from the stomach, but that rate of absorption is not as great as occurs in the intestine. In this study the rate of absorption through the intestinal wall was about twice as great as in the stomach. The data do indicate, however, that the rate of absorption through the intestinal wall is related to the surface area of the intestine. An approximate 2-fold increase in the length of the intestinal segment resulted in about a 2-fold increase in rate of disappearance of fluoride from the lumen of the intestine. This would be expected if a diffusion process were operating in the absorption of fluoride.

Data are presented to strengthen further the evidence that an active transport system is not involved in absorption of fluoride in the rat. Rate of diffusion was measured at 3 different temperatures. If an active transport system were in operation, one would have expected the diffusion of fluoride to be temperature dependent. However, comparable rates of diffusion were found at all 3 temperatures studied. Similarly, data are presented which represent the rate of fluoride diffusion in the presence of agents known to poison enzyme systems. Once again the rate of diffusion in the absence of any poison was quite comparable to that obtained in the presence of the 3 poisons investigated. Collectively, these data suggest that no active transport is involved in removal of fluoride from the gastrointestinal tract of the rat and further suggest that the mechanism involved may be one of simple diffusion. The values obtained in these studies compare favorably with the rates of fluoride absorption in the rat indicated by earlier *in vivo* studies.

Summary. In vitro studies were conducted on gastrointestinal segments from albino rats in an attempt to elucidate factors involved in the mechanism of fluoride absorption. The results of these studies suggested that no ac-

tive transport system is involved since fluoride would not travel against a concentration gradient across the intestinal wall nor was the diffusion rate influenced appreciably by enzyme poisons or temperature changes. In addition, the rate of diffusion varied with the diffusion area as was expected and diffusion was inhibited by physiological concentrations of calcium and magnesium.

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Preparation of Water Insoluble Thrombin.* (28897)

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The preparation of water insoluble proteolytic enzymes such as trypsin(1) and papain (2) has been successfully accomplished. It is possible to remove such enzymes quantitatively and rapidly from reaction mixtures by physical means such as filtration and centrifugation. This permits the investigator to use these insoluble enzymes in the study of intermediate steps in progressive proteolytic reactions. The study of blood clotting has been hampered by the difficulty of isolating intermediate reactions. A central component of the clotting process, thrombin, not only clots fibrinogen, but also appears to accelerate the early stages of its own formation in an autocatalytic manner. We therefore felt that an insoluble thrombin would be particularly advantageous in the study of clotting processes involving activation or degradation of other clotting factors. We have prepared such a water insoluble thrombin by coupling soluble thrombin with a copolymer of p-amino-DL-phenylalanine and L-leucine, and report here some of its properties.

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Materials and methods. Preparation of insoluble thrombin. Bovine thrombin (thrombin-topical, Parke-Davis & Co.) was chromatographed on Amberlite, C-G-50 type II by modification of the method of Rasmussen (3). The inactive protein was washed off the column with 0.1 M sodium phosphate buffer pH 7.5. The thrombin was then eluted by 0.3 M sodium phosphate buffer pH 8.0 containing 1 M sodium chloride. The active effluent was concentrated by dialyzing against 10% polyvinylpyrrolidone for 24 hours followed by dialysis against 0.154 M NaCl for 4-6 hours at 4°C. This concentrated thrombin had a specific activity of 665 NIH units/mg protein as compared to the starting material, 57 NIH units/mg protein.

A copolymer of p-amino-DL-phenylalanine and L-leucine was prepared at the Weizman Institute of Science, Rehoboth, Israel by the method of Bar-Eli and Katchalski(1).† This copolymer has a molar ratio of L-leucine and p-amino-DL-phenylalanine of 2.1:1. Coupling of the thrombin to the copolymer was

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