small amounts of calf aorta extracts or the mucopolysaccharide, chondroitin sulfate A are added to the cultures. The lipid clearing effect noted in cells is often associated with stimulation of cellular growth and with a process of lipid dilution by cellular multiplication. However, in the case of one aortic extract, lipid clearing took place without significant stimulation of cellular growth. The latter suggests a specific cellular clearing effect as distinguished from lipid dilution by cell division and growth.

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Role of Skeleton and Kidney in Fluoride Absorption in Rat. (28367)

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Considerable work has been done in an attempt to identify those factors which regulate or modify the absorption of fluoride from the gastro-intestinal tract. Several different inorganic elements, such as calcium(1, 2), magnesium(3,4), phosphorus(5), and molybdenum(6-8), may alter the rate of fluoride absorption. Metabolism studies have suggested further that the age of the animal(9), especially with respect to rate of skeletal growth, and its previous exposure to fluoride affect fluoride absorption(10). The metabolic equilibrium suggested by Fig. 1 indicates that if the kidneys are damaged or seriously impaired, thus removing a major source of fluoride detoxification, fluoride which is absorbed must either be deposited in the skeleton or remain in the soft tissues and circulating body fluids. Carlson and co-workers (11) have shown in the nephrectomized rat using isotopically-labeled fluoride that the tissue fluoride concentration is elevated. Since it has been shown that the metabolism of fluoride may be significantly affected in renal failure(12), and an increase in soft tissue fluoride content results in nephrectomized rats, these studies were conducted to provide more information concerning the specific efforts of nephrectomy and previous exposure to fluoride upon rate of fluoride absorption in the rat.

Experimental. Series I. A total of 18 male Wistar strain albino rats ranging in age from 55 to 60 days were divided into 2 groups of 8 each and a third group of 2 animals according to body weight. All animals were housed in pairs in an air-conditioned room and received a low-fluoride stock corn

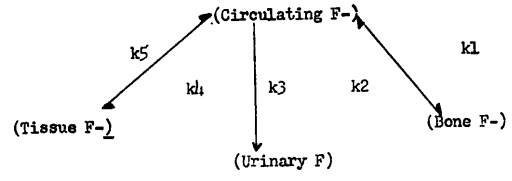

FIG. 1. Schematic diagram of metabolic pathways of fluoride.

diet (F = 0.5 μg/g) and distilled fluoride-low water (F = 0.05 μg/ml) *ad libitum* prior to the study. The animals were starved 24 hours prior to experiment. The animals in Group I were bilaterally nephrectomized under ether anesthesia and the renal vessels ligated. The incision was surgically closed and the animals allowed 10 minutes to revive from anesthesia. Group II animals were not operated and served as controls. The animals in both groups were given 1.2 mg of fluoride as aqueous sodium fluoride. One-half of the animals in Groups I and II were sacrificed after 2 hours by chloroform inhalation, and the remainder after 4 hours. The animals in Group III which were not operated and received no fluoride were sacrificed, and their entire gastrointestinal tract removed and analyzed for fluoride to provide control data. Surgical and chemical techniques employed have been described(6). The gastrointestinal tract of all groups was exposed, ligated at the esophageal-epigastric junction and at the sigmoid colon, and the ligated portion removed intact. The intact segments were then placed in separate tared, fused-silica crucibles, covered with a slurry of redistilled water containing exactly 1.0 g of purified low-fluoride calcium oxide, and evaporated to dryness under infra-red lamps. The sample was charred, and ashed for 6 hours at 600°C in a muffle furnace. The sample and the dish were weighed, the sample carefully pulverized and mixed, and an aliquot of the ash analyzed for fluoride by perchloric acid distillation and thorium nitrate titration according to procedures described previously(2).

Series II. A total of 48 young Wistar rats approximately 40 days of age, raised from our stock colony, were divided equally into 4 groups according to sex and body weight. Groups A and B were controls and were used during the portion of the study involving measurement of the rate of fluoride absorption. These animals were maintained on the same low-fluoride diet used in Series I. Groups C and D were given 1.0 mg of fluoride as aqueous NaF daily by stomach tube for a period of 125 days. At this period of the study Groups A and B, which were being continued in the study without receiving fluoride, were to be considered essentially unexposed to fluoride while Groups C and D had each been "exposed" to 125 mg of fluoride. Groups A and C were now subdivided into groups of 6 each, while the animals in Groups B and D were continued on the same fluoride regimen for an additional 125 days.

For convenience the animals in Group A are subdivided into Groups A1 and A2. The animals in A1 were unoperated and served as unexposed, unoperated controls while those in A2 were bilaterally nephrectomized and served as unexposed, operated controls. The animals in Group C were similarly divided into C1 which was unoperated and provided an unoperated but previously fluoride-exposed group, while those in C2 were bilaterally nephrectomized and provided a previously fluoride-exposed and nephrectomized group of animals. After 125 experimental days the animals in Group A and C were starved for 24 hours and then given 1.0 mg of fluoride as aqueous NaF. After 4 hours all animals were anesthetized and their entire gastrointestinal tracts removed and analyzed for fluoride as described in Series I.

The animals in Groups B and D were continued on the respective control and 1.0 mg F regimens for another 125 days when they were similarly operated and examined for fluoride absorption as described above.

Data. The data obtained in Series I are shown in Table I. The gastrointestinal tracts of the control animals contained an average of 567 and 373 μg of fluoride after 2 and 4 hours, respectively. These data indicate that control rats, as employed in this study, absorb 52.8% of the administered fluoride during the first 2 hours and a total of 68.9% during the 4-hour period. In contrast, only 30.6 and 40.2% of the administered fluoride was absorbed after 2 and 4 hours, respectively, in the bilaterally nephrectomized rats, with both differences significant at the .001 probability level.

The data for the Series II studies are shown in Tables II and III. Table II shows that 4 hours after administration of 1.0 mg

TABLE I. Effect of Bilateral Nephrectomization on Fluoride Absorption in Young Rats (Series I).

Treatment	Absorption period (hr)	Avg net* μg F found	"t" value	P value	Avg % absorption
Control	2	567 ± 32†			52.8
"	4	373 ± 38			68.9
Nephrectomy	2	832 ± 13	11.67	.001	30.6
"	4	718 ± 27	10.62	.001	40.2

* Value remaining after deducting 13.1 μg F found in G.I. tracts of animals which received no fluoride.

† Standard deviation.

of fluoride to animals which had been on the study plan for 125 days but which received no fluoride, their gastrointestinal tracts contained an average of 357 μg of fluoride, indicating that 64.3% of the administered fluoride was absorbed. The gastrointestinal tracts from the animals in Group A2 which had no previous exposure to fluoride but were bilaterally nephrectomized were found to contain 581 μg of fluoride, indicating that 41.9% of the administered fluoride was absorbed during the same time period in the same age animal living in the same environment. These data indicate that in adult animals having no previous exposure to fluoride complete removal of both kidneys significantly ($p = .001$) decreases fluoride absorption during the 4-hour study period by 34.8%.

The control animals in Group C1 which had a previous fluoride exposure of 125 mg of fluoride but were unoperated absorbed 50.2% of the administered fluoride. Comparison of these data with those for the Group A1 animals indicates that the previous exposure to 125 mg of fluoride significantly ($p = .001$) decreased the amount of fluoride absorbed during the 4-hour period by 21.9%. The gastrointestinal tracts in the bilaterally nephrectomized animals in Group C2 which had a similar exposure to fluoride contained 567 μg of the administered fluoride indicating an absorption of 43.3%. Comparison of this value with that obtained in the previously exposed but unoperated animals in Group C1 indicates that bilateral nephrectomization, even in previously exposed animals, resulted in a 13.7% decrease ($p = 0.1$) in amount of fluoride absorbed during the 4-hour period. However, com-

TABLE II. Effect of Bilateral Nephrectomization and Previous Exposure to 125 mg of Fluoride upon Fluoride Absorption (Series II, Groups A and C).

Group	Previous fluoride exposure	Treatment	Avg net μg F found	"t" value	P value	Avg % absorption
A 1	Control	Control	357 ± 23*	—	—	64.3
A 2	"	Neph	581 ± 12	15.68	>.001	41.9
C 1	Fluoride	Control	498 ± 45	5.86	>.001	50.2
C 2	"	Neph	567 ± 16	15.23	>.001	43.3

* Standard deviation.

TABLE III. Effect of Bilateral Nephrectomization and Previous Exposure to 250 mg of Fluoride upon Fluoride Absorption (Series II, Groups B and D).

Group	Previous fluoride exposure	Treatment	Avg net μg F found	"t" value	P value	Avg % absorption
B 1	Control	Control	361 ± 54*	—	—	63.9
B 2	"	Neph	622 ± 29	8.89	>.001	37.8
D 1	Fluoride	Control	501 ± 98	2.85	>.03	49.9
D 2	"	Neph	777 ± 34	17.51	>.001	22.3

* Standard deviation.

parison of the data obtained in Groups A2 and C2 indicates that in bilaterally nephrectomized animals, this amount of skeletal saturation of previous exposure to fluoride has no appreciable effect ($p = 0.2$) upon the amount of fluoride absorbed during the 4-hour experimental period.

The data obtained from the animals in Groups B and D which had been exposed to 250 mg of fluoride are shown in Table III. Those animals in Group B1 which had no previous exposure and were unoperated absorbed 63.9% of the fluoride administered during the absorption period. The gastrointestinal tracts of the animals in Group B2, which were nephrectomized immediately prior to administration of the single dose of fluoride but which had no previous exposure to fluoride, contained 622 μg of fluoride indicating that 37.8% of the administered fluoride had been absorbed. These data again indicate that nephrectomy significantly ($p = .001$) decreased the rate of fluoride absorption, in this instance by a value of nearly 41%.

The data obtained in Groups D1 and D2 indicate the absorption rates in animals of the same age but which were exposed to 250 mg of fluoride prior to determination of the rate of fluoride absorption. The gastrointestinal tracts of the fluoride-exposed control animals in Group D1 contained an average of 501 μg of fluoride, indicating that 49.9% of the test dose of fluoride was absorbed. Comparison of the value obtained in Group B1 indicates that previous exposure to 250 mg of fluoride decreases the rate of fluoride absorption in rats by 21.9%, significant at the 0.03 probability level. The gastrointestinal tracts of the animals in Group D2 which were previously exposed to 250 mg of fluoride and were bilaterally nephrectomized contained an average of 777 μg of fluoride indicating an absorption of 22.3%. Comparison of this value with that obtained in Group D1 indicates that even with previous exposure to fluoride, bilateral nephrectomy reduces the rate of fluoride absorption, ($p = .001$) although the combination of nephrectomization and previous exposure did not completely inhibit fluoride absorption. Comparison of the values obtained in Groups B2 and D2 again shows that previous exposure to fluoride in nephrectomized rats significantly ($p = .001$) decreases the rate of fluoride absorption by 41.0%.

Discussion. Several investigators have suggested that absorption of fluoride may be altered appreciably by a number of physiological factors. The data reported here suggest that in weanling rats, complete removal of the kidneys, thus eliminating a major pathway for removal of fluoride from circulating body fluids, significantly decreased the absorption of fluoride, and suggest the possibility that concentration of fluoride in the circulating fluids may play a vital role in fluoride absorption. Several workers have suggested that there is a greater possibility of fluoride toxicity in subjects having kidney damage in that more fluoride is retained by the skeleton. These data obtained here suggest that the body may have a protective mechanism against the possibility of such fluoride toxicity by decreasing the rate of fluoride absorption in the gastrointestinal tract in subjects having imperfect renal physiology. This hypothesis remains to be validated under various experimental conditions.

Previous exposure of the skeleton to fluoride is thought by some workers to decrease the subsequent rate of its retention by the skeleton. The data reported here suggest that, if an animal receives previous fluoride, less fluoride is absorbed upon its subsequent administration than in animals which have had no previous exposure to fluoride. This effect is even more pronounced after nephrectomy, in that even less of the fluoride is absorbed from the gastrointestinal tract. These data thus suggest that if the kidney is no longer able to remove fluoride from the circulating body fluids, the process of fluoride absorption is partially reversed and the body reacts by absorbing less fluoride from the GI tract into the circulating body fluids.

Summary. Two studies were conducted in an attempt to learn more concerning the effect of bilateral nephrectomy and previous exposure to fluoride upon rate of fluoride absorption in the rat. The first study was conducted in weanling animals having no previ-

ous exposure to fluoride and indicated that bilateral nephrectomy significantly decreased the rate of fluoride absorption by 41.9% during the first 2 hours after fluoride administration and by 41.7% 4 hours thereafter. In the second study, this effect was substantiated using animals exposed to 2 different levels of fluoride. Previous exposure to 125 mg of fluoride decreased the rate of fluoride absorption by 21.9% and when the animals were in addition nephrectomized, a further decrease of 13.7% was noted. After exposure to 250 mg of fluoride, the rate of fluoride absorption was decreased by a similar value of 21.9%. Nephrectomy further decreased the rate of fluoride absorption and the combination of previous exposure and nephrectomy decreased the rate of fluoride absorption by more than 65%. These data suggest that when kidney function becomes impaired the body responds by decreasing the rate of fluoride absorption.

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Radiochromium-Labeled Lymphocytes in the Rat.* (28368)

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The life cycle of lymphocytes has been investigated by transfusion of blood from patients with chronic lymphocytic leukemia (1), by counting lymphocytes, obtained from the appendix, after injection into rabbits(2), by measuring the volume of myeloid and lymphoid tissue in the rat and counting the number of mitotic figures(3), by studying parabiotic rats(4), by collecting lymph and counting the lymphocytes of dogs(5), cats (6), rats(7) and rabbits(8,9), and by labeling lymphocytes with a fluorescent dye(10).

With the advent of radioisotopes, lymphocytes labeled with phosphate-P^{32} were studied(11,12). More recently McCall and associates(13) and Shorter and Bollman(14) studied lymphocytes labeled with radiochromium (Cr^{51}), Schooley and Berman(15) studied tritiated lymphocytes from the thoracic duct, and Gowans(16,17) studied tritiated-DNA labeled lymphocytes from the thoracic duct.

Despite these studies, clear answers are not yet available to the questions: How long does the lymphocyte live? How long does it remain in the bloodstream? Is there a lymph-to-blood-to-lymph circulation? The blood span is reported as 30 minutes to 12 hours, the life span 3 hours to many days, and whether or not there is recycling of the lymphocytes is disputed. This paper describes part of an extensive consideration (18) of these questions.

Methods. Labeling rat lymphocytes. Intestinal lymph was obtained from Sprague-Dawley rats, weighing 180 to 290 g, by the method of Bollman and associates(19). The

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