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Radiofluoride Distribution in Tissues of Normal and Nephrectomized Rats.* (25542)

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The beneficial effect of the fluoride ion upon incidence of dental caries has stimulated interest in the metabolism of fluoride both in calcified and in non-calcified tissues. The kidney has been of particular interest because it is the major route of excretion of absorbed fluoride. The amount of fluoride in non-calcified tissues has been difficult to evaluate analytically because it is found in minute quantities (micromoles/kg). However, development of the crystal scintillation counter has allowed application of radiotracer technics to certain facets of soft tissue fluoride metabolism. The most useful radioisotope of fluoride is F^{18} which is a positron emitter with a half-life of 109.7 minutes(1).

Procedure. Each of 4 male rats (A-D) was given a 1 ml intraperitoneal injection of radiofluoride in a saline solution containing 0.2 ppm stable fluoride. Two other animals (E,F) were each given a 2 ml injection of the solution. Three of the animals (A,C,E), were heminephrectomized 10 days before the experiment, and the remaining kidney was removed on the morning of the experiment. Each animal was sacrificed 80 minutes after injection. The soft tissues, digested to solution in 5 N NaOH on a steam bath, were diluted to a 25 ml volume. The humeri were dissolved by shaking them in dilute HCl. Radiofluoride contents of the tissues were determined by using a well-type crystal scintillation counter to measure radioactivity in a 10 ml aliquot of the dissolved tissue. These measurements were made over 8-minute periods and were corrected for background count, volume and radioactive decay to one point in time. Chloride contents were determined by the wet ash procedure described by Caster, MacDonald and Armstrong(2). Water contents of the fresh tissues were evaluated by loss in weight on drying at 100°C.

Results. Table I presents in 3 ways the mean radiofluoride concentrations found in the soft tissues of normal and nephrectomized animals. These data (Columns II and III) show an elevation of the absolute radiofluoride concentration in the tissues of the animals deprived of kidney function. However, no marked change in the pattern of soft tissue distribution of fluoride occurred as a result of nephrectomy. This fact is best seen from Table II in which radiofluoride concentration in the water of the soft tissues is compared with that of the plasma water. This ratio of fluoride concentration does not appear to differ in normal and nephrectomized animals. The tissues can be divided (Table II) into 4 groups: 1) Brain tissue had lowest relative radiofluoride concentration. 2) Tail tendon was the only soft tissue which had a radiofluoride concentration greater than plasma water. 3) Heart muscle and skeletal muscle had a radiofluoride concentration close to one-half the concentration present in plasma water. 4) Skin, liver and testicular tissue showed a range of concentrations, when compared to plasma water concentrations, with a high of 0.90 for skin tissue water and a low of 0.68 for liver tissue water.

Table I also gives tissue concentrations of radiofluoride in counts per microequivalent

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TABLE I. Mean Radiofluoride Concentrations in Selected Tissues of Normal and Nephrectomized Rats.

I	II		III		IV		V	
	F^{18} cts/g tissue (wet wt)		F^{18} cts/g tissue H_2O		F^{18} cts/μeq Cl		F^{18} cts/μeq Cl in tissue / F^{18} cts/μeq Cl in plasma	
Tissue	Normal	Nephr.	Normal	Nephr.	Normal	Nephr.	Normal	Nephr.
Plasma	8,902	11,275	9,624	12,189	78	101	1.0	1.0
Muscle	3,661	5,982	4,882	6,710	223	328	2.9	3.3
Liver	4,565	5,013	6,360	8,414	165	287	2.1	2.9
Tendon	11,087	13,502	18,098	21,785	165	186	2.1	1.9
Heart	3,625	4,098	4,696	5,308	108	122	1.4	1.2
Testes	5,539	4,600*	7,614	5,921*	103	84*	1.3	.9*
Skin	5,160	6,828	8,703	10,472	83	116	1.1	1.2
Brain	1,199	2,198	1,537	2,819	34	58	.4	.6

* Lower radiofluoride concentrations in testes of nephrectomized animals were a result of the operative procedure which compromised testicular blood flow because of the anatomical relationships of renal and testicular vasculature(3).

of chloride. In Column V ratios of the radiofluoride concentrations in the tissues, so expressed, to that of the plasma is presented. When radiofluoride contents of the tissues are expressed thus, *i.e.*, in comparison with the predominantly extracellular chloride anion, these ratios are a measure of the comparative magnitude of fluid volumes through which the ions are distributed. On the premise that chloride is located in extracellular fluid a ratio greater than one indicates penetration of fluoride into the intracellular fluid. This result was most conspicuous in skeletal muscle; also, in tendon and liver the ratio was greater than one. Skin, heart muscle and testicular tissues showed values of radiofluoride per unit of chloride which were close to that of plasma. Brain tissue had the lowest ratio, always less than one. This finding indicates a very low fluoride concentration in brain or an efficient barrier to migration and exchange of fluoride.

The soft tissues and plasma of the nephrectomized animals showed only a slight increase in fluoride concentration without any marked change in pattern of fluoride distribution among the tissues in spite of the fact that the nephrectomized animals, in contrast to the normal animals, were prevented from disposing of some fluoride in the urine. The extra radioisotope in the nephrectomized animals was sequestered in the skeleton (Table III) with only moderate increases in amounts present in the soft tissues. The results with nephrectomized animals E and F are a good example of this process since these animals received a double dose of fluoride.

Discussion. Previous work has shown that peak radiofluoride concentrations in soft tissues occur well within 80 minutes after a single dose of the radioisotope(4). Therefore, this period of time is adequate to obtain maximum ionic penetration. Assuming that both chloride and fluoride ions are not concentrated

TABLE II. Ratio of Counts per Minute per ml of Water in Tissue Sample Divided by Counts per Minute per ml of Plasma Water..

	Normal B	Nephr. A	Normal D	Nephr. C	Normal F	Nephr. E	Normal Mean	Nephr. Mean
Plasma	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Tendon	2.04	1.47	2.11	2.13	1.43	1.76	1.86	1.78
Skin	.85	.83	1.03	.88	.83	.86	.90	.86
Testes	.82	.52*	.58	.45*	.97	.48*	.79	.48*
Liver	.69	.56	.60	.74	.74	.77	.68	.69
Muscle	.44	.45	.54	.61	.56	.60	.51	.55
Heart	.49	.43	.47	.46	.50	.41	.49	.43
Brain	.20	.23	.14	.32	.13	.18	.17	.23

* See footnote to Table I.

TABLE III. Radiofluoride Contents of Rat Humeri.

Normal animals	B	D	F
F^{18} cts/min./g humerus (wet wt)	311,762	425,342	376,572
Nephrectomized animals	A	C	E
F^{18} cts/min./g humerus (wet wt)	523,538	459,377	916,589

at sites such as cell membranes then relative amounts of these ions found in a tissue is a measure of the fluid volume available to the ion in the tissue.

The sequestration of fluoride by skeletal structures is well known. It is also well known that tendonous structures can be sites of deposition for calcified mineral. It is possible that the unusual concentration of fluoride in the tendon is associated with this same phenomenon. However, chloride concentrations in tendon water are also high and it is possible that the relatively high fluoride and chloride concentrations in tendon water are due to a loss of water through evaporation during exposure and dissection of the thread-like strands of tendon.

Summary. 1) Radiofluoride given by intraperitoneal injection was absorbed and distributed throughout fluid compartments of the rat. It was found in concentrations exceeding that of plasma in the skeleton and in the tail tendon. In all other soft tissues examined, concentration in tissue water was some fraction of plasma concentration. Tissue concentrations in nephrectomized animals were increased but the pattern of distribution was not changed. The skeleton sequestered the bulk of the radiofluoride which would have been excreted by the kidneys. 2) The radiofluoride content of tissues expressed in terms of chloride content of the tissues varied with the nature of the tissue. It was lowest in the brain, highest in skeletal muscle, and was increased in most tissues of nephrectomized animals. The soft tissue fluid compartment volumes are not the same for the 2 halogen anions fluoride and chloride.

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Studies in Leukemia: XIV. Effect of Human Serum on Development of Leukemia in AKR Mice.† (25543)

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An antiserum that will prevent induction of leukemia in C3H and Swiss mice can be produced in rabbits by using cell-free filtrates made from the brains of leukemic mice or patients who died of leukemia(1). Experiments were undertaken to ascertain whether a similar antiserum could be prepared in human volunteers. Acceleration of development of leukemia in the AKR mouse by inoculation of cell-free filtrate from human brain(2) served as the experimental model.

Materials and methods. Equal parts of the brains of 4 patients who died of acute leukemia (2 lymphoblastic, one myeloblastic, one monoblastic) were pooled, and a 1.0% suspension was prepared. A cell-free filtrate was made of this as previously described(3). The filtrate was rapidly frozen and stored at

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